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DAIRY SCIENCES - CURRENT TECHNOLOGICAL TRENDS AND FUTURE PERSPECTIVES

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Research Based Book Chapter

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RESEARCH BASED BOOK CHAPTER

IMPACT OF CURD WASHING AND BETA CYCLODEXTRIN ON THE REDUCTION OF CHOLESTEROL CONTENTS IN EWE´S MILK CHEESE

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Abstract

Beta-cyclodextrin (β-CD) is a cyclic oligosaccharide composed of seven glucose units. The utilization of β-cyclodextrin (β-CD) in the field of food research has been on the trend, primarily for its ability to reduce cholesterol levels. This can be attributed to β-CD's strong attraction to nonpolar compounds, including cholesterol. The objective of this study was to assess the impact of curd washing in ewe's milk cheese on the reduction of cholesterol by β-CD in pasteurized ewe's milk Manchego cheese, specifically focusing on the primary constituents of milk, lipids, and flavor characteristics. A significant reduction of approximately 98.45% in cholesterol levels was observed in the experimental cheeses subjected to treatment with β-CD. The remaining β-CD exhibited significant differences ($p \leq 0.05$) between the curd washing and non-curd washing conditions, with values of 0.51% and 0.27%, respectively. The physicochemical properties, specifically fat, moisture, and protein content, were not observed to be significantly affected by the process of curd washing, regardless of the presence or absence of β-CD. However, slight differences were observed in the levels of soluble nitrogen and non-protein nitrogen because of the treatment. The lipid fraction of fatty acids, triglycerides, and phospholipids exhibited comparable quantities in both the treated and untreated cheese samples, as influenced by curd washing and the presence or absence of β-CD. The influence of β-CD on flavor compounds and short chain free fatty acids was not found to be statistically significant for most compounds. However, 3 methyl butanal and ethanol were the only compounds that exhibited a statistically significant difference ($p \leq 0.05$). There were no statistically significant differences ($p \leq 0.05$) observed in the sensory attributes, including flavor, aroma, color, and acceptability, between the curd washing treatments with or without β-CD. However, a significant difference ($p \leq 0.05$) was observed in texture between the two treatments. The β-CD molecules are edible and nontoxic in nature, making them suitable for use in the safe processing of cholesterol removal in cheese manufacturing.

The reduction of residual β-CD can be improved by implementing curd washing, resulting in a 47% decrease. Hence, the current investigation points out that the implementation of curd washing proved to be an efficacious technique for the reduction of cholesterol in Manchego cheese while maintaining its inherent qualities. The methodology employed for the treatment of β-cyclodextrin (β-CD) was implemented.

Keywords

Beta Cyclodextrin, Ewe´s Milk, Curd Washing, Cheese, Manchego, Lipids

1. Introduction

Dairy products are considered nutrient-dense and healthy foods for all ages of consumers; however, dairy products such as butter, cream and cheeses possessed high fat contents. The occurrence of different metabolic diseases has been associated with lipids types and cholesterol level in the diet. During last decade, the American Heart Association and World Health Organization have recommended the consumers to follow the dietary guidelines regarding intake of saturated lipids, fatty acids and cholesterol to lower the risk of metabolic disorders and related coronary heart diseases. Therefore, the demand for dairy products with low cholesterol contents has been increasing rapidly because of health-conscious perceptions [1, 2]. In response, the dairy industry professionals and researchers are emphasizing to manufacture the dairy products with decreased level of cholesterol. Moreover, the high demand of cholesterol-reduced dairy products, coupled with the minimum production cost, is a salient feature that has been focused to aid dairy alternatives suppliers and manufacturers to target this dairy market.

Different strategies and methods to develop foods having reduced cholesterol contents are in practice on commercial, laboratory and industrial scales. Promising techniques for cholesterol reduction include blending in vegetable oils [3, 4], adsorption with digitonin and saponin [5, 6], extraction by crystallization and distillation [7, 8], removal by supercritical carbon dioxide extraction [9, 10] and assimilation of cholesterol by microbial enzymes [11, 12]. Several research and review published studies have described the use of β-CD in food [13 - 15]. β-CD molecule being non-toxic and non-digestible can be safely used as molecule to bind and remove cholesterol effectively from milk and dairy products [15]. From structural point of view, the β-CD molecule is a

cyclic oligosaccharide consisting of 7 glucose units. Majorly, enzyme cyclodextrin glycotransferases act on starch and produced β-CD molecule. This process involves breakdown of the polysaccharides chain and alternatively produced cyclic polysaccharide molecules. The doughnut shaped β-CD molecule has central portion with characteristics circular hydrophobic space and has similarity with cholesterol molecule diameter which provides the unique opportunity to β-CD molecule for its affinity to non-polar molecules such as cholesterol [16, 17].

The research investigations have concluded the feasibility of β-CD molecule to be applied as excellent substance for cholesterol removing from foods and dairy products including milk, however, up to date limited information is available which explained the impact of β-CD application for cholesterol reduction in ewe´s milk Manchego cheese and physico-chemical properties. Region wise, the Manchego cheese is considered one of the most famous representatives of the Spanish hard cheeses. Manchego cheese is commercially manufactured in the Spanish region of La Castilla la Mancha and substrate used for production is pure ewe´s milk which is collected from local herds under suitable conditions regulated by an origin appellation [18]. Manchego cheese is abundant with fat contents which ranged higher than 50% in the dry cheese. Manchego is a rich flavor cheese which increases with the aging time. The main mandate of this study was to evaluate the impact of curd washing on the reduction of cholesterol in ewe´s milk cheese and β-CD retention in treated pasteurized ewe´s milk Manchego cheese. Moreover, investigation was focused for β-CD effect on the main components of milk, lipids, and flavor characteristics of regular Manchego cheese.

2. Materials and Methods

2.1. Experimental Chemicals

Sigma (St. Louis, MO) provided the chemicals such as a-cyclodextrin (a-CD), β-cyclodextrin (β-CD) and all other reagent grade for the lab experiments. Deionized water was obtained from water purification and filtration system (Millipore Co).

2.2. Manchego Manufacturing Process

The Alonso method [15] was followed to treat ewe's milk with 1% beta cyclodextrin. The method described by Fernández-Garca [19] was used to make Manchego cheese. The same amount of whey that was drained off was used to make two separate batches of curd washing with deionized water. Three months of storage were allowed for cheese to be matured and ripened.

2.3. Gross Chemical Composition

The recommended procedures were used to determine the composition of fat, moisture, protein and nitrogen fractions [20].

2.4. Beta Cyclodextrin Determination

The analysis of β-CD was conducted using the Alonso method [21]. 10 g of cheese and 5 mg of a-CD was mixed in 1 mL water which served as the internal standard for quantitative analysis. Following a two-minute agitation period at a temperature of 40°C, the mixture underwent centrifugation at ambient temperature for a duration of thirty minutes, with a rotational speed of 40000 revolutions per minute (rpm). This process was conducted to eliminate the uppermost layer and subsequently filter it through a 0.45 m Millipore Co. membrane. A 30 µL portion of the supernatant, which had been spiked with the internal standard, was transferred to the autosampler. In the context of high-performance liquid chromatography (HPLC) analysis, a volumetric quantity of 10 µL of the supernatant aliquot was introduced onto the chromatographic column. Empower 2, a chromatographic data software manufactured by (Waters, Milford, MA) was used for the data collection and analysis processes. To complete the HPLC study and data acquisition, a Water (Alliance 2695 separation module) system was coupled to a 410 refractive index (RI) detector. A YMC ODS-AQ column manufactured by Teknochroma, USA was used in the separation process. The mobile phase was made by adding methanol to water at a 7:93 ratio. A steady flow of 1 mL/min of mobile phase was then injected into the system under isocratic conditions. The time required for elution was calculated using water standards. The concentration of β-CD in the

experimental sample was then quantified by comparing the peak area of the experimental sample containing β-CD to the internal standard (α-CD).

2.5. *Lipid Extraction Method*

The extraction of lipids from experimental samples was conducted according to the International Standard Method for Milk and Milk Products [22]. In summary, the experimental procedure adopted the addition of an ammonia-ethanol solution to the test component, subsequently followed by a lipid extraction technique utilizing hexane and diethyl ether as extraction solvents. The solvent underwent complete evaporation upon removal of the top layer. The lipid extracts were collected and subsequently stored in amber glass vials at a temperature of -20°C by following a nitrogen flushing procedure.

2.6. *Cholesterol Determination*

The technique employed for the analysis of cholesterol was capillary gas chromatography (GC), utilizing direct injection of milk fat, as outlined by Alonso [23]. For the GC analysis, a solution was prepared by dissolving approximately 30 mg of anhydrous milk fat and an internal standard of 5-cholestane (3.5 mg/mL in hexane) in 1 mL of hexane. The resulting solution (0.5 µL) was then injected into GC for analysis. The direct method for gas chromatography (GC) analysis of free cholesterol was applied using the Agilent Technology 6890 chromatograph (Palo Alto, CA). The GC apparatus was well equipped with a flame ionization detector and HP-5 fused silica capillary column. The dimensions of the column were 30m x 0.32 mm i.d. and 0.25 µm thickness. The experimental chromatography was conducted under the following conditions: The carrier gas was maintained at a head pressure of 17 psi. The column temperature was initially set at 280 °C and was held constant for 1 minute. Subsequently, the temperature was increased to 355 °C at a rate of 3 °C per minute. The injector temperature was set at 350 °C, while the detector temperature was set at 360 °C. The process of peak identification involved comparing the relative retention times of the peaks with the standard retention times. The quantification of cholesterol was conducted by evaluating the peak area of the sample in relation to the specific internal standard (5-

cholestane). The calculation of the cholesterol reduction percentage in milk fat was determined by employing the formula given below:

[(100 – cholesterol in milk fat) x 100]/ amount of cholesterol in untreated milk)

2.7. Triglycerides and Fatty Acids Analysis

Fatty acids methyl esters (FAMES) were produced by employing a solution of 2 N KOH in methanol to methanolized the extracted lipids. Alkaline catalysts were utilized in this process. The Fatty Acid Methyl Esters (FAMES) were analyzed using an Agilent Technology 6890 chromatograph (Palo Alto, California) well equipped with flame ionization detector (FID). The separation of fatty acids was conducted utilizing the Alonso [24] method on a CP-Sil 88 fused-silica capillary column (50 m x 0.25 mm i.d. x 0.2 m film thickness, Chrompack, California, USA). The triglycerides were assessed using gas chromatography (GC) with direct injection, utilizing a flame ionization detector and an Agilent gas chromatograph 6890 (Palo Alto, CA). The experiments were conducted following the Alonso method [24], by application of a WCOT fused silica capillary column with dimensions of 25 m x 0.25 mm x 0.1 m film thickness and coated with coated with OV 17 TRI (J.W. Scientific, Polson, CA, USA) [25].

2.8. Phospholipids Analysis

The extraction of cheese fat was conducted using an Accelerated Solid Extraction ASE-200 extractor (Dionex Corp., Sunnyvale, CA). A total of 2 g of freeze-dried cheese sample and 2 g of sea sand were utilized for the extraction process. The stainless-steel extraction cell employed in this study was coated with filters on both sides. A solvent mixture consisting of dichloromethane and methanol in a volumetric ratio of 2:1 was used, while a fixed condition of 10.3 MPa pressure was maintained to achieve the maximum yield of cheese fat [26]. The lipid classes were separated using a high-performance liquid chromatography (HPLC) system (model 1260; Agilent Technologies Inc.) connected to an evaporative light scattering detector (SEDEX 85 model; Sedere SAS, Alfortville Cedex, France). The nebulizing gas used was prefiltered compressed air, applied at a pressure of 350 kPa and a temperature of 60°C, with the gain was set to 3. In this study, two columns were applied in a sequential manner. The first column utilized

was with dimensions (250 × 4.5 mm Zorbax Rx-SIL column with 5-µm particle diameter; Agilent Technologies Inc.) while a second as precolumn with identical packing material was also utilized [26].

2.9. Volatile Compounds Analysis

The volatile fraction was analyzed using the headspace gas chromatographic-mass spectrometric (GC-MS) method as described by Alonso [27]. A total volume of 80 microliters of an aqueous solution containing propionic acid ethyl methyl ester at a concentration of 1.14 milligrams per milliliter was combined with 10 grammes of anhydrous sodium sulphate, which served the purpose of water retention. This mixture was then added to 10 grammes of pre-homogenized cheese. Before utilization, every individual standard dilution in an aqueous solution was prepared and placed in vials that were tightly sealed to prevent any external contamination. The vials were stored at a temperature of ¬20°C. The samples were subjected to a temperature of 80°C for a duration of 60 minutes to establish thermodynamic equilibrium between the sample and gaseous phase. Subsequently, the samples were analyzed using a static headspace apparatus (Model HSS 19395; Hewlett-Packard). The experimental setup was adjusted to apply pressure for a duration of 5 seconds, followed by a stabilization and filling period of 18 seconds, and finally an injection period lasting two minutes. Helium was employed as the carrier gas at a flow rate of 17.5 mL/min. The determination of volatile compounds involved the application of a Hewlett-Packard GC Model 5890 which was connected to a selective MS Model 5972. Polyethylene glycol was used as the injection medium in split mode (7:1 split ratio, 18 rate) for introducing samples into a capillary silica column (HP Innovas, 60m length, 0.25mm inner diameter, 0.25 m film thickness, manufactured by Hewlett Packard). The carrier gas used in the experiment was helium, with a flow velocity of 18 36.5 cm/s. The temperature protocol employed for the column involved an initial temperature of 33°C for a duration of 5 minutes, followed by a gradual increase of 1°C per minute until reaching 38°C. Subsequently, the temperature was further increased at a rate of 7°C per minute until reaching 210°C, where it was maintained for a period of 10 minutes. The interface line of the mass spectrometer (MS) was heated to a temperature of 280

degrees Celsius, and the injection process was carried out at a temperature of 200 degrees Celsius. The voltage of the photomultiplier was measured to be 18 V, while the electronic ionization energy was found to be 70 eV. Additionally, the electronic ionization energy was determined to be 1647 V.

2.10. Short Chain Free Fatty Acids Determination

To assess the existence of short chain free fatty acids (SCFFA), a 1g cheese sample was subjected to homogenization in 20 mL of distilled water. Subsequently, the resulting mixture was centrifuged at a speed of 10,000 revolutions per minute for a duration of 10 minutes. Finally, the supernatant was filtered using a 0.40 mm filter. The investigation was conducted utilizing a capillary silica column on a Hewlett-Packard model 5890 A gas chromatograph equipped with a flame ionization detector. The specific column used was an HP FFAP with dimensions of 30m length, 0.25mm internal diameter, and a 0.25 m film thickness, manufactured by Agilent Technologies J & W. The identification of each free fatty acid (FFA) was accomplished by utilizing the retention length of a standard, while quantitative analysis was conducted by comparing the peak areas of individual FFAs with the peak areas of 2-ethyl butanoic acid, which was employed as an internal standard.

2.11. Sensory Evaluation

A group of twenty-two expert sensory panelists conducted an evaluation of cheeses that were assigned random tags. The attributes of flavor, aroma, color, texture and acceptability were assessed using a five-point scale, with ratings ranging from 1 (poor) to 5 (excellent).

2.12. Statistical Analysis

SAS software (version 8.02, SAS Institute Inc, Cary, NC, USA) was used to run an analysis of variance (ANOVA) on the experimental data. A student t-test was used to analyze the data, and differences between treatments were declared significant at a P value of less than 0.05.

3. Results and Discussion

3.1. Gross Chemical Composition

The gross chemical composition and cholesterol removal rate are shown in Table 1 for four cheeses: control cheese without β-CD (CC), experimental cheese with 1% of β-CD (EC), washed control cheese without β-CD in milk (WCC) and washed experimental cheese with 1% of β-CD in milk (WEC). The fat, moisture, and protein content exhibited comparable ratios between the CC-EC and WCC-WEC, specifically ranging between 34.50% - 34.13% versus 32.51% - 31.53% for fat content, 37.79% - 38.10% versus 37.15% - 36.46% for moisture content, and 25.68% - 24.11% versus 25.10% - 24.96% for protein content, respectively. The cholesterol-reduced cheese is lower in fat than the control because less fat is integrated with casein through a fat protein network. This is probably owing to β-CD's effect on the casein matrix [28].

Comparisons of CC-EC and WCC-WEC showed statistically significant differences in soluble nitrogen (SN) and non-protein nitrogen (NPN) levels ($P \leq 0.05$). The percentages of protein content were found to be 25.68% and 24.11% for CC-EC, and 25.10% and 24.96% for WCC-WEC, respectively. Similarly, the percentages of protein content were 4.76% and 4.88% for CC-EC, and 5.79% and 6.01% for WCC-WEC, respectively. This phenomenon may be attributed to a slight elevation in proteolysis observed in EC cheeses, suggesting a potential rise in peptidase activity resulting from the influence of the β-CD [29]. Proteolysis occurs throughout the process of ripening, serving as a crucial metabolic process that regulates the sensory characteristics. Insoluble caseins undergo partial conversion into polypeptides and amino acids. The application of β-CD to milk, which serves as the precursor for cheese production, leads to alterations in the casein structure, thereby influencing the levels of soluble nitrogen (SN) and non-protein nitrogen (NPN). Additionally, this process may potentially expedite the maturation phase of the cheese.

The cholesterol removal rate in the WEC group, as compared to the WCC group, demonstrated a significant reduction of 98.45% versus 2.02%, respectively. This reduction was observed by comparing the cholesterol levels of 195.67 mg/100g fat to 191.71

mg/100g fat in the WEC group, and 1.37 mg/100g fat to 1.12 mg/100g fat in the WCC group. In a study conducted by Kwak [30], it was observed that cholesterol removal from Cheddar cheese exhibited similarities to the findings of Kin [31] in blue cheese, both of which utilized β-CD. There was a statistically significant difference ($p < 0.05$) observed in the remaining β-CD content between the EC and WEC samples, with values of 0.51% and 0.27%, respectively. This study provides confirmation that the removal of cholesterol using β-CD and curd washing does not significantly alter the primary chemical composition of Manchego ewe's milk cheese.

Table 1 Gross chemical composition for the experimental Manchego cheeses

Parameter	CC	EC	WCC	WEC
Fat (%)	34.50 ± 1.12^{a}	34.13 ± 1.25^{a}	32.51 ± 1.18^{a}	31.53 ± 1.13^{a}
Moisture (%)	37.79 ± 1.65^{a}	38.10 ± 1.80^{a}	37.15 ± 1.93^{a}	36.46 ± 1.70^{a}
Protein (%)	25.68 ± 1.04^{a}	24.11 ± 1.12^{a}	25.10 ± 1.16^{a}	24.96 ± 1.05^{a}
SN (% as protein)	4.76 ± 0.23^{a}	4.88 ± 0.29^{a}	5.79 ± 0.32^{b}	6.01 ± 0.35^{b}
NPN (% as protein)	2.41 ± 0.19^{a}	2.66 ± 0.22^{a}	3.95 ± 0.24^{b}	4.22 ± 0.41^{b}
pH	4.87 ± 0.15^{a}	5.28 ± 0.19^{a}	4.85 ± 0.25^{a}	5.30 ± 0.21^{a}
Cholesterol (mg/100g fat)	195.67 ± 6.03^{a}	191.71 ± 5.31^{a}	1.37 ± 0.19^{a}	1.12 ± 0.14^{a}
Cholesterol removal (% fat)	-	2.02 ± 0.25^{a}	97.29 ± 4.56^{b}	98.45 ± 5.12^{b}
Remain BCD (%)	-	-	0.51 ± 0.13^{a}	0.27 ± 0.11^{b}

CC: Control cheese; EC: experimental cheese (1% β-CD); WCC: Washed control cheese; WEC (1% β-CD): Washed experimental cheese. β-CD: Beta-cyclodextrin.

SN: Soluble nitrogen (% as protein); NNP: Non protein nitrogen (% as protein); β-CD: Beta-cyclodextrin.

$^{a, b}$ Different letters in the same row mean significant differences ($p \leq 0.05$). Mean standard deviation (n=3).

3.2. Lipid Characteristics

The average percentages of fatty acids in several cheese samples are presented in Table 2. These samples include control cheese made without β-CD in the milk (referred to as CC), experimental cheese made with 1% of β-CD (referred to as EC), washed control cheese made without β-CD in the milk (referred to as WCC), and washed experimental cheese made with 1% of β-CD in the milk (referred to as WEC). The amounts of specific fatty acids in fat derived from different sources did not change significantly ($p \leq 0.05$). There is a scarcity of literature regarding research conducted on the impact of β-CD on the lipid composition during the manufacturing process of low cholesterol cheeses. The fatty acid composition of the fractionated milk fat differed significantly from the control cheeses in a study by Chen [32] that used supercritical fluid extraction with carbon dioxide to remove cholesterol and fractionate milk fat. The authors found that the amounts of short and medium chain fatty acids in the extracted milk fat were reduced by 40% and 10%, respectively, when compared to the levels in the control milk fat. The results obtained by Gonzalez were consistent [9]. The purpose of this study was to compare the effects of β-CD treatment on cholesterol removal and the effects of β-CD treatment on the percentages of short-chain (C4-C8), medium-chain (C10-C12), and long-chain (C14-C18) fatty acids. Similar results were seen in a study on the use of beta cyclodextrin to lower cholesterol levels in milk fat by Alonso [15].

Triglyceride composition of washed control cheese (WCC) and washed experimental cheese (WEC) with 1% concentration of β-CD in milk are presented in Table 3, along with the control cheese (CC) that had no β-CD in the milk as a comparison. Fatty cheese's triglycerides were broken down into 15 categories, based on their carbon chain lengths (from C26 to C54). Each cluster stands for the accumulated existence of several triglyceride molecular variants sharing the same carbon-atom count. There were no significant differences between the CC-EC and WCC-WEC groups at the 0.05 level of significance. The short-term range (C24-C32) exhibits a slight difference in values between two intervals, specifically (8.81 - 8.93%) compared to (8.18 - 8.19%). Similarly, the medium-term range (C34-C48) shows a marginal variation in values,

namely (77.63 - 78.15%) versus (77.51 - 77.99%). Lastly, the long-term range (C50-C54) demonstrates a slight discrepancy in values, with (9.31 - 9.29%) in contrast to (9.95 - 9.98%). To date, there is a limited of research examining the triglyceride composition of cheeses subjected to β-CD treatment for cholesterol removal. The potential variation in triglyceride composition may have arisen from the differential extraction efficiency of solvents employed in the extraction process, as well as the utilization of supercritical fluid extraction techniques by the researchers.

Table 2 Fatty acids composition (g/100 g fat) of the experimental Manchego cheeses

Fatty acid	CC	WCC	EC	WEC
C4:0	2.24 ± 0.19^{a}	2.18 ± 0.22^{a}	2.14 ± 0.26^{a}	2.16 ± 0.24^{a}
C6:0	1.74 ± 0.06^{a}	1.68 ± 0.08^{a}	1.68 ± 0.05^{a}	1.67 ± 0.05^{a}
C8:0	1.70 ± 0.05^{a}	1.66 ± 0.07^{a}	1.66 ± 0.08^{a}	1.64 ± 0.06^{a}
C10:0	5.02 ± 0.15^{a}	4.95 ± 0.18^{a}	4.95 ± 0.13^{a}	4.91 ± 0.16^{a}
C10:1	0.28 ± 0.03^{a}	0.25 ± 0.05^{a}	0.25 ± 0.07^{a}	0.24 ± 0.05^{a}
C12:0	3.19 ± 0.11^{a}	3.14 ± 0.16^{a}	3.14 ± 0.18^{a}	3.11 ± 0.14^{a}
C14:0	9.22 ± 0.84^{a}	9.21 ± 0.92^{a}	9.21 ± 0.51^{a}	9.14 ± 0.42^{a}
C14:1	0.90 ± 0.03^{a}	0.88 ± 0.08^{a}	0.86 ± 0.06^{a}	0.89 ± 0.04^{a}
C15:0	0.24 ± 0.02^{a}	0.25 ± 0.04^{a}	0.25 ± 0.05^{a}	0.23 ± 0.07^{a}
C16:0	27.16 ± 1.52^{a}	27.41 ± 1.13^{a}	27.41 ± 1.18^{a}	27.21 ± 1.11^{a}
C16:1	0.73 ± 0.12^{a}	0.77 ± 0.15^{a}	0.77 ± 0.17^{a}	0.71 ± 0.13^{a}
C17:0	0.54 ± 0.07^{a}	0.58 ± 0.09^{a}	0.58 ± 0.07^{a}	0.52 ± 0.05^{a}
C18:0	13.39 ± 0.55^{a}	13.59 ± 0.65^{a}	13.59 ± 0.52^{a}	13.56 ± 0.39^{a}
C18:1t	2.62 ± 1.13^{a}	2.65 ± 0.26^{a}	2.65 ± 0.23^{a}	2.78 ± 0.14^{a}
C18.1c	23.28 ± 0.35^{a}	22.93 ± 1.06^{a}	22.93 ± 1.16^{a}	22.78 ± 1.21^{a}
C18:2	3.39 ± 0.08^{a}	3.26 ± 0.31^{a}	3.26 ± 0.24^{a}	3.26 ± 0.18^{a}
C18:3	0.39 ± 0.08^{a}	0.40 ± 0.06^{a}	0.40 ± 0.05^{a}	0.39 ± 0.03^{a}
C18.2 (c9t11)	0.96 ± 0.06^{a}	0.97 ± 0.08^{a}	0.97 ± 0.06^{a}	0.97 ± 0.05^{a}

CC: Control cheese; EC: experimental cheese (1% β-CD); WCC: Washed control cheese; WEC (1% β-CD): Washed experimental cheese. β-CD: Beta-cyclodextrin.

$^{a, b}$ Different letters in the same row mean significant differences ($p \leq 0.05$). Mean standard deviation (n=3).

Table 3 Triglycerides composition (g/ 100 g fat) of the experimental Manchego cheeses

Fatty acid	CC	EC	WCC	WEC
C24	0.33 ± 0.06[a]	0.35 ± 0.04[a]	0.32 ± 0.08[a]	0.31 ± 0.06[a]
C26	0.88 ± 0.09[a]	0.81 ± 0.08[a]	0.80 ± 0.05[a]	0.77 ± 0.08[a]
C28	1.64 ± 0.15[a]	1.53 ± 0.17[a]	1.44 ± 0.13[a]	1.42 ± 0.17[a]
C30	2.42 ± 0.23[a]	2.58 ± 0.27[a]	2.47 ± 0.21[a]	2.50 ± 0.29[a]
C32	3.54 ± 0.40[a]	3.66 ± 0.46[a]	3.15 ± 0.39[b]	3.01 ± 0.45[b]
C34	4.89 ± 0.38[a]	4.88 ± 0.41[a]	5.04 ± 0.48[a]	5.09 ± 0.56[a]
C36	7.21 ± 0.66[a]	7.35 ± 0.52[a]	7.04 ± 0.54[a]	7.18 ± 0.50[a]
C38	10.66 ± 1.11[a]	10.51 ± 1.19[a]	10.65 ± 1.30[a]	11.01 ± 1.39[a]
C40	17.35 ± 1.32[a]	17.60 ± 1.42[a]	17.89 ± 1.32[a]	17.15 ± 1.42[a]
C42	16.02 ± 1.40[a]	16.32 ± 1.35[a]	16.17 ± 1.50[a]	16.21 ± 1.68[a]
C44	8.83 ± 0.77[a]	8.66 ± 0.71[a]	7.44 ± 0.66[a]	8.89 ± 0.51[a]
C46	7.14 ± 0.62[a]	7.21 ± 0.63[a]	7.04 ± 0.52[a]	7.16 ± 0.59[a]
C48	5.35 ± 0.55[a]	5.62 ± 0.59[a]	5.71 ± 0.49[a]	5.30 ± 0.54[a]
C50	4.28 ± 0.35[a]	4.35 ± 0.42[a]	4.39 ± 0.51[a]	4.55 ± 0.62[a]
C52	4.57 ± 0.39[a]	4.44 ± 0.44[a]	4.31 ± 0.56[a]	4.24 ± 1.57[a]
C54	4.78 ± 0.43[a]	4.66 ± 0.36[a]	4.58 ± 0.45[a]	4.60 ± 0.41[a]

CC: Control cheese; EC: experimental cheese (1% β-CD); WCC: Washed control cheese; WEC (1% β-CD): Washed experimental cheese. β-CD:Beta-cyclodextrin.

[a, b] Different letters in the same row mean significant differences ($p \leq 0.05$). Mean standard deviation (n=3).

Phospholipid profiles for four types of cheese as shown in Table 4 were control cheese made with milk that lacked β-CD (CC), experimental cheese made with 1% β-CD (EC), washed control cheese made with milk that lacked β-CD (WCC) and washed experimental cheese made with 1% β-CD (WEC). No statistically significant difference ($p \leq 0.05$) in the relative composition of the different phospholipid classes in relation to total phospholipids (PL) was found between the groups (CC-EC vs WCC-WEC) of cheeses using analysis of variance. Phosphatidylethanolamine (PE) exhibited the highest abundance among the phospholipids, with a range of 42.42% to 45.72% as a proportion of total phospholipids (PL). This was followed by phosphatidylcholine (PC), which ranged from 27.23% to 32.04% as a proportion of total PL. Sphingomyelin (SM) had a comparatively lower abundance, ranging from 26.70% to 27.84% as a proportion

of total PL. Alonso (15) conducted a study investigating the impact of β-CD on phospholipids found in pasteurized milk and obtained comparable findings.

The collective proportion of these three distinct phospholipid species exceeded 80% of the overall phospholipid content found in dairy products. One possible explanation for the lack of impact of β-CD on these constituents of milk fat may be attributed to the formation of an inclusion complex between β-CD and cholesterol. The hydrophobic nature of the core cavity of β-CD is responsible for its affinity towards nonpolar substances such as cholesterol. The dimensions of the cavity are such that it closely accommodates a cholesterol molecule, highlighting the highly specific capability of β-cyclodextrin to create an inclusion complex with cholesterol. Consequently, β-CD can be readily accessed in the aqueous phase, where they form insoluble inclusion complexes that can be separated through centrifugation [15].

Table 4 Phospholipids composition of the experimental Manchego cheeses

Phospholipids	CC	WCC	EC	WEC
Total PLs	0.12 ± 0.03[a]	0.05 ± 0.01[a]	0.11 ± 0.03[a]	0.09 ± 0.01[a]
PE	42.42 ± 4.05[a]	45.72 ± 5.12[a]	38.25 ± 1.40[a]	40.61 ± 1.53[a]
PI	1.93 ± 1.31[a]	2.95 ± 0.30[a]	2.46 ± 0.62[a]	2.53 ± 1.51[a]
PS	1.75 ± 0.53[a]	1.68 ± 0.35[a]	3.21 ± 1.94[a]	2.10 ± 1.31[a]
PC	27.23 ± 0.74[a]	32.04 ± 1.40[a]	31.04 ± 2.21[a]	29.20 ±1.80[a]
SM	26.70 ± 5.32[a]	27.84 ± 3.83[a]	25.20 ± 1.53[a]	25.62 ± 3.22[a]

CC: Control cheese; EC: experimental cheese (1% β-CD); WCC: Washed control cheese; WEC (1% β-CD): Washed experimental cheese. β-CD; Beta-cyclodextrin.

PLs: Phospholipids; PE: Phosphatidylethanolamine; PI: Phosphatidylinositol; PS: Phosphatidylserina; PC: Phosphatidylcoline; SM: Sphyngomyelin.

[a, b] Different letters in the same row mean significant differences ($p \leq 0.05$). Mean standard deviation (n=3).

3.3. Flavor Characteristics

Table 5 shows the extracted flavor compounds from four cheeses: control cheese (CC) made without the addition of β-CD in milk; experimental cheese (EC) made with 1% β-CD in milk; washed control cheese (WCC) made without β-CD; and washed experimental cheese (WEC) made with 1% β-CD in milk. After conducting a thorough examination, scientists were able to isolate 13 individual flavor compounds from both cheese samples, with some of those chemicals being more prevalent in one or the other. There was a total of 13 different flavor compounds found in cheeses, including 5 ketones, 3 aldehydes, and 6 alcohols. There were no statistically significant differences ($p \leq 0.05$) between the CC-EC and WCC-WEC cheeses when comparing the total amounts of ketones [(2567.00 - 2348.54) vs (2307.91 - 2141.87 ppm)], aldehydes [(1139.63 - 1062.10 ppm) vs (1377.45 - 1472.90 ppm)] and alcohols [(4217.77 - 4200.14 ppm) vs (4516.80 - 4590.10 ppm)] between (CC-EC vs WCC-WEC) cheeses. The levels of 3 methyl butanal in the CC-EC cheese ranged from 1121.42 to 1045.60 ppm, while the levels in the WCC-WEC cheese ranged from 4385.30 to 4456.02 ppm, representing a statistically significant difference ($p \leq 0.05$). Notably, among the evaluated flavor components, ethanol generation was highest, correlating with the results obtained by Kwak [30] in Cheddar cheese treated with β-CD. Researchers [33] found that the overall flavor components of cream cheese were not significantly different after the cholesterol was removed using β-CD. Odd-numbered-carbon ketones have low sensory thresholds and distinctive smell characteristics. Fatty acids undergo beta-oxidation and decarboxylation to produce the compounds. Aldehydes are quickly converted into alcohols or their equivalent acids, hence they are not the principal ingredients in cheeses. An aminotransferase catabolizes branched-chain amino acids, resulting in branched-chain aldehydes such 3-methyl butanal [34]. When compared to CC cheese and ethanol, this chemical showed statistical significance ($p \leq 0.05$) in the setting of EC.

Table 6 displays the levels of SCFFAs (acetic acid, propionic acid, butyric acid, and caproic acid) in a variety of cheeses. The samples include control cheese without the addition of cyclodextrin (β-CD) referred to as CC, experimental cheese containing 1% cyclodextrin referred to as EC, control cheese that underwent a washing process

without the addition of cyclodextrin referred to as WCC, and experimental cheese that underwent a washing process with 1% cyclodextrin referred to as WEC. The cheese samples were obtained from milk. After a three-month maturation period, the total short-chain fatty acid (SCFFA) levels in the matured cheeses did not exhibit a significant difference ($p \leq 0.05$). The average SCFFA levels were found to be 149.14 - 147.13 ppm and 151.77 - 158.43 ppm, respectively. Additionally, the individual levels of SCFFAs did not show any significant variation. Similar findings were reported by [30, 35] regarding the levels of short-chain fatty acids (SCFFAs) generated in both the control and cholesterol-reduced procedures, as well as in cheddar cheese manufactured using β-CD. During the three-month ripening period, the release of butyric and caproic acid occurs, contributing to the development of the characteristic flavour profile of Manchego cheese.

Table 7 presents the sensory characteristics of four cheese samples: control cheese without β-CD (CC) in milk, experimental cheese with 1% of β-CD (EC), washed control cheese without β-CD (WCC), and washed experimental cheese with 1% of β-CD (WEC). The sensory attributes were evaluated on a scale of 1 to 5. There were no statistically significant differences ($p \leq 0.05$) observed in the flavour [(3.32 - 3.16) vs (2.97 - 2.86)], aroma [(3.59 - 3.12) vs (2.88 - 2.80)], colour [(3.69 - 3.61) vs (3.49 - 3.37)], and acceptability [(3.45 - 3.39) vs (3.22 - 3.18)] between the CC-EC and WCC-WEC cheeses. The texture of the cheeses (CC-EC vs WCC-WEC) exhibited a significant difference ($p \leq 0.05$), with values of (3.45 - 3.39) and (3.22 - 3.18), respectively. This disparity may be attributed to the higher proteolysis observed in the EC and WEC cheeses resulting from the treatment with β-CD. Additionally, a slight increase in moisture content was observed in the cheese treated with β-CD, leading to a slower drainage process [36]. During a three-month ripening period, there was consistent adherence to the overall preference, with no observed differences in terms of flavor, aroma, color, or acceptability between the (CC - EC) and (WCC - WEC) groups. The present study revealed that, although there were slight differences, most sensory characteristics and overall preferences exhibited similar patterns between the control group and the curd washed with β-CD for a duration of three months. Consequently, it is possible to

hypothesize about the potential to produce cholesterol-reduced Manchego cheese through the implementation of β-CD and curd washing techniques.

Table 5 Volatile compounds (ppm) of the experimental Manchego cheeses

Compounds	CC	EC	WCC	WEC
Ketones				
2 Propanone	420.38 ± 32.39^{a}	401.66 ± 28.36^{a}	381.05 ± 26.89^{a}	369.96 ± 25.32^{a}
2 Butanone	27.65 ± 4.51^{a}	23.32 ± 4.16^{a}	20.16 ± 4.21^{a}	21.45 ± 4.69^{a}
2,3 Butanodione	1271.54 ± 48.45^{a}	1123.60 ± 53.75^{a}	1145.81 ± 56.38^{a}	1041.65 ± 59.98^{a}
2 Heptanone	562.30 ± 29.49^{a}	535.42 ± 25.12^{a}	512.18 ± 22.78^{a}	496.21 ± 30.21^{a}
3 Hydroxy 2 butanone	286.12 ± 18.66^{a}	264.54 ± 17.65^{a}	248.70 ± 20.09^{a}	212.60 ± 24.60^{a}
Aldehydes				
3 Methyl butanal	1121.42 ± 48.32^{a}	1045.69 ± 65.12^{a}	1358. 96 ± 70.32^{b}	1452.95 ± 77.80^{b}
Hexanal	14.16 ± 6.50^{a}	12.56 ± 3.89^{a}	13.54 ± 4.09^{a}	15.74 ± 4.96^{a}
Nonanal	4.05 ± 1.21^{a}	3.85 ± 1.09^{a}	4.95 ± 1.19^{a}	4.21 ± 1.29^{a}
Alcohols				
2 Propanol	14.50 ± 3.56^{a}	12.96 ± 4.39^{a}	10.64 ± 3.70^{a}	9.65± 3.96^{a}
Ethanol	4107.60 ± 62.30^{a}	4085.50 ± 89.60^{a}	4385.30 ± 95.79^{b}	4456.02 ± 109.10^{b}
2 Methyl 1 propanol	43.18 ± 7.11^{a}	46.13 ± 6.45^{a}	55.66 ± 7.80^{a}	58.69 ± 7.02^{a}
2 Butanol	29.31 ± 6.85^{a}	22.45 ± 5.02^{a}	25.69 ± 5.56^{a}	23.20 ± 5.04^{a}
2 Heptanol	36.18 ± 6.04^{a}	33.10 ± 7.32^{a}	39.57 ± 6.12^{a}	42.54 ± 5.52^{a}

CC: Control cheese; EC: experimental cheese (1% β-CD); WCC: Washed control cheese; WEC (1% β-CD): Washed experimental cheese. β-CD: Beta-cyclodextrin.

SN: Soluble nitrogen (% as protein); NNP: Non protein nitrogen (% as protein); β-CD: Beta-cyclodextrin.

$^{a, b}$ Different letters in the same row mean significant differences ($p \leq 0.05$). Mean standard deviation (n=3).

Table 6 Short chain free fatty acids (SCFFA) (ppm) of the experimental Manchego cheeses

SCFA	CC	EC	WCC	WEC
Acetic acid	92.91 ± 7.19[a]	93.06 ± 7.68[a]	193.06 ± 6.19[a]	129.53 ± 8.96[a]
Propionic acid	35.28 ± 5.65[a]	34.64 ± 4.56[a]	35.36 ± 4.96[a]	36.02 ± 4.90[a]
Butyric acid	17.10 ± 3.96[a]	16.13 ± 3.06[a]	17.32 ± 3.60[a]	21.16 ± 3.93[a]
Caproic acid	3.85 ± 2.52[a]	3.30 ± 2.80a	3.96 ± 3.12[a]	3.72 ± 3.61[a]

CC: Control cheese; EC: experimental cheese (1% β-CD); WCC: Washed control cheese; WEC (1% β-CD): Washed experimental cheese. β-CD: Beta-cyclodextrin.

SN: Soluble nitrogen (% as protein); NNP: Non protein nitrogen (% as protein); β-CD: Beta-cyclodextrin.

[a, b] Different letters in the same row mean significant differences ($p \leq 0.05$). Mean standard deviation (n=3).

Table 7 Sensory analysis of the experimental Manchego cheeses

Attribute	CC	EC	WCC	WEC
Flavour	3.32 ± 0.44a	3.16 ± 0.67a	2.97 ± 0.89a	2.86 ± 0.71a
Aroma	3.59 ± 0.49a	3.12 ± 0.57a	2.88 ± 0.83a	2.80 ± 0.81a
Color	3.69 ± 0.68a	3.61 ± 0.63a	3.49 ± 0.73a	3.37 ± 0.86a
Texture	3.70 ± 0.57a	3.43 ± 0.51a	3.29 ± 0.72b	3.03 ± 0.83b
Acceptability	3.45 ± 0.60a	3.39 ± 0.73a	3.22 ± 0.76a	3.18 ± 0.75a

CC: Control cheese; EC: experimental cheese (1% β-CD); WCC: Washed control cheese; WEC (1% β-CD): Washed experimental cheese. β-CD: Beta-cyclodextrin.

SN: Soluble nitrogen (% as protein); NNP: Non protein nitrogen (% as protein); β-CD: Beta-cyclodextrin.

[a, b] Different letters in the same row mean significant differences ($p \leq 0.05$). Mean standard deviation (n=3).

4. Conclusions

The goal of this research was to examine the effects of curd washing on the essential components of milk, lipids, and flavor characteristics of a regular Manchego cheese after cholesterol was reduced by β-CD in pasteurized ewe's milk Manchego cheese. Cholesterol was lowered by about 98.45 percent in β-CD-treated experimental cheeses. Curd washing had a statistically significant impact on the residual β-CD (0.51 vs. 0.27%, $p \leq 0.05$). Fat, moisture, and protein were not affected by curd washing with or without β-CD, and only soluble nitrogen and nonprotein nitrogen showed minor modifications by treatment. Curd washing and the presence or absence of β-CD had no discernible effect on the relative levels of fatty acids, triglycerides, and phospholipids in the lipid fraction of the treated and untreated cheese. Flavoring chemicals and free fatty acids with a short chain length were unaffected by the β-CD. The only two substances that showed statistical significance ($p \leq 0.05$) were 3-methylbutanal and ethanol. Sensory attributes (taste, smell, color, and acceptability) were not affected by curd washing with or without β-CD, but texture was (p 0.05). The β-CD molecules can be used safely as a cholesterol elimination processing during cheese production, lowering the amounts of residual β-CD, even though they are edible and harmless. As a result, the current investigation revealed that the treatment of β-CD by the action of curd washing was an effective procedure for removing cholesterol while keeping the qualities of Manchego cheese.

Author Contributions

Analysis design, L.A. and J.F.; performing the experiment, L.A., M.V.C., and J.F.; writing, L.A and J.F; review and editing, L.A. M.V.C., and J.F.

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Conflicts of Interest

The authors declare no conflict of interest.

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DAIRY SCIENCES - CURRENT TECHNOLOGICAL TRENDS AND FUTURE PERSPECTIVES

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Sustaining the Dairy Sector in Pakistan: Challenges and Strategies for Growth

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REVIEW BASED BOOK CHAPTER

Sustaining the Dairy Sector in Pakistan: Challenges and Strategies for Growth

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Abstract

This chapter critically evaluates the significant challenges encountered by the dairy industry in Pakistan between 2013 and 2023. Despite being a leading dairy powerhouse, boasting expansion and improvements over the last decade, the industry has faced numerous substantial obstacles that have hindered its progress. This chapter provides an in-depth examination of these constraints and their subsequent impact on the industry's performance, profitability, and competitive environment. It anticipates and addresses potential future challenges, such as antibiotics and Aflatoxins, considerations for ß-casein protein, introducing dairy animals with high genetic potential, and drivers of operating costs. These findings offer a rich reservoir of knowledge for policymakers, industry leaders, and researchers in this sector. The chapter also proposes potential approaches to these challenges to improve the industry's outlook.

Keywords
Dairy Industry, Milk Production, Milk Consumption, Future Challenges, Supply Chain, Livestock Breeds, Dairy Issues

1. Introduction

Pakistan's agricultural economy thrives on the critical contribution of the dairy sector, which has noticeably catalyzed substantial growth in agricultural output. Firmly embedded as a cornerstone in the economic structure, this industry offers financial assistance, vital income streams for rural residents, and ample job opportunities [1]. Striking figures from the Pakistan Bureau of Statistics indicate that the livestock sector, including dairy, will constitute roughly 60% of total agricultural production by the year 2020 [2]. Worldwide, Pakistan holds the fourth position in dairy production, as evidenced by its significant consumption of fresh milk, comprising 97% of total intake, while the

remaining 3% is pasteurized milk [3]. Recent government intervention underscores the significance of dairy farming, supporting small farmers' livelihoods [4]. Among over 8 million rural households engaged in livestock, small-scale farmers grapple with challenges linked to shifting climate [5]. Addressing the need for increased animal protein, dairy development projects target enhanced productivity through crossbreeding with high-yielding varieties [6].

This chapter is dedicated to identifying the challenges encountered by the dairy industry in Pakistan from 2013 to 2023 and proposing potential solutions to these issues. The objective is to highlight the primary restrictions that have hampered the country's dairy industry's growth and development over this period. This chapter will also provide an assessment of the current situation of the dairy business and highlight potential possibilities for future expansion.

2. General Characteristics of Dairy Farming

In the context of Pakistan, the private sector predominantly participates in urban and rural dairy farming across varying scales. However, the prevailing perception of the sector portrays it as fragmented and oriented towards subsistence. Except for a handful of peri-urban establishments, the bulk of dairy production occurs within integrated crop-livestock systems. Existing research primarily classifies farms based on geographical location and herd magnitude, delineating four key principal milk production systems: smallholder subsistence, smallholder market-oriented, rural commercial, and peri-urban [7]. They are described in the sections that follow:

2.1. Smallholder subsistence system

Rural locales harboring micro-farmers devoid of significant milk market access contribute milk at an economically frugal rate tailored to familial requisites. An archetypical subsistence entity encompasses a trinity of buffalo specimens. Eliciting an average bovine milk yield of 3 liters daily, this modality routinely hinges on non-monetary assets, notably kin-owned land, and labor, as pivotal constituents underpinning dairy production endeavors within these households. This category encapsulates 70% of smallholder milk producers [8].

2.2. Smallholder market-oriented system

In this context, farmers possess direct access to a dairy market, fostering a proclivity for heightened production beyond familial needs. The elemental structure of this production scheme entails a family-owned collective of five buffaloes. This assembly comprises a male calf, one or potentially two female followers, and three mature female specimens [9]. Milk is marketed through numerous routes, through direct sales to nearby local shops, and transactions with intermediaries or agents employed by milk-handling businesses [10].

2.3. Rural commercial system

Milk is brought twice daily to the market. The principal expenditures within this framework involve hired labor, animal housing, veterinary care, animal feed, water supply, electricity expenditures, and the cost of milk transportation. Milk is supplied directly to municipal retailers or through annual contracts with intermediaries. Recent breakthroughs within the dairy sector have spurred sure forward-thinking farmers to amplify their investments in milk production. However, these progressive initiatives represent a mere fraction, touching only 1% of United States dairy farms [11]. On average, milk yield per cow is 10 liters each day.

2.4. Peri-urban system

Peri-urban production unfolds within expansive marketable enterprises on the fringes of key urban centers. These establishments maintain conservation herds spanning a range of ten to 200 head, typifying an average of 50 animals. Predominantly, these herds comprise 90 percent buffalo and 10 percent cows [12]. Milk transportation to the market transpires twice daily. The milk supply mechanism encompasses two avenues: direct provision to local municipal retailers or execution via yearly contractual agreements with intermediaries [13]. A visual representation of milk production across diverse manufacturing systems is illustrated in Figure 1.

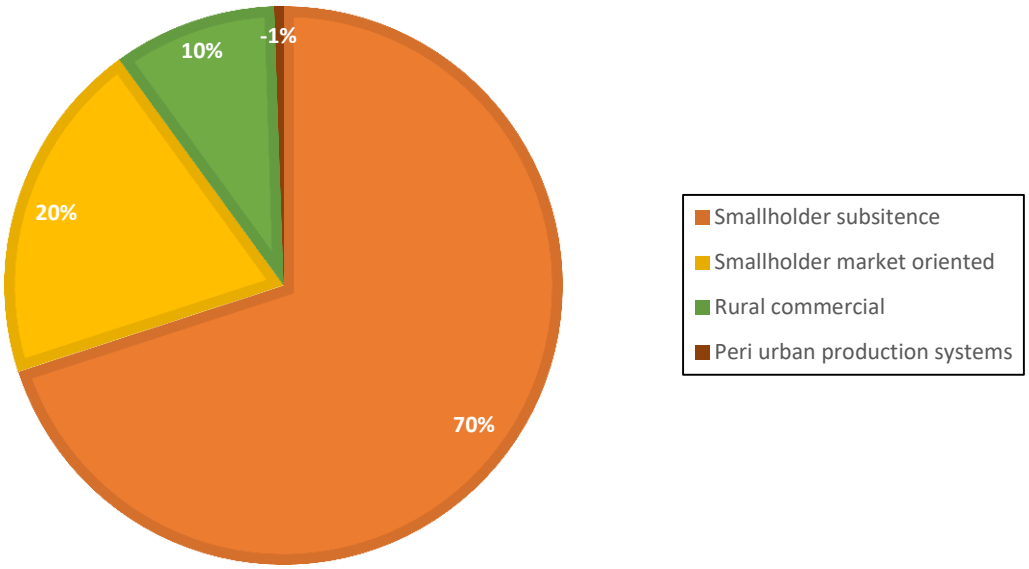

Figure 1: Milk production from various manufacturing systems

3. Trends in Milk Production

The provided Table 1 offers insights into the milk output across various animal species, represented in thousand tons, spanning the years from 2013-14 to 2021-22. The total milk production increased from 50,990 to 65,745 thousand metric tonnes during this time. More cow and buffalo milk contributed to the majority of this increase. Goat and sheep milk remained the same, but camel milk slightly increased, as shown in Table 1, while Figure 2 graphically illustrates the anticipated milk output from 2013-2023.

4. Trends in Milk Consumption

Table 2 delineates human milk consumption in thousand tons and the corresponding milk consumption of various livestock species for each year from 2013-22. Human milk consumption witnessed a gradual upsurge, ascending from 41,133 thousand tons in 2013–14 to 52,996 thousand tons. The graphic depiction of estimated human milk consumption is presented in Figure 3.

Table 1: The expected milk production for the years 2013-22 in 000 tonnes

Species	2013-14	2014-15	2015-16	2016-17	2017-18	2018-19	2019-20	2020-21	2021-22
Cow	18,027	18706	19412	20143	20,903	21,691	22,508	23,357	24,238
Buffalo	31,252	32,180	33137	34122	35,136	36,180	37,256	38,363	39,503
Sheep	38	38	39	39	40	40	41	41	42
Goat	822	945	867	891	915	940	965	991	1018
Camel	851	862	873	885	896	908	920	932	944
Milk Gross Production	50,990	52,632	54,328	56,080	57,890	59,759	61,690	63,684	65,745

Source: An Economic Survey of Pakistan (2013-2022)

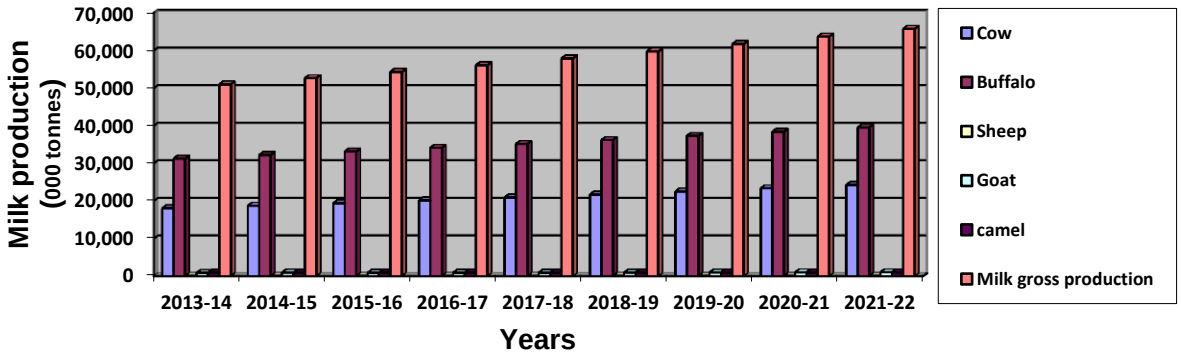

Figure 2: A graphical illustration of projected milk output for the years 2013-22
Source: Pakistan Economic Survey

Table 2: The estimated human milk consumption from 2013 to 22 000 tonnes

Species	2013-14	2014-15	2015-16	2016-17	2017-18	2018-19	2019-20	2020-21	2021-22
Cow	14,421	14,956	15,529	16,115	16,722	17,353	18,007	18,686	19,390
Buffalo	25,001	25,744	26,510	27,298	28,109	28,944	29,805	30,691	31,603
Sheep	38	38	39	39	40	40	41	41	42
Goat	822	845	867	891	915	940	965	991	1018
Camel	851	862	873	885	896	908	920	932	944
Milk (Human Consumption)	41,133	42,454	43,818	45,227	46,682	48,185	39,737	51,340	52,996

Source: An Economic Survey of Pakistan (2013-2022)

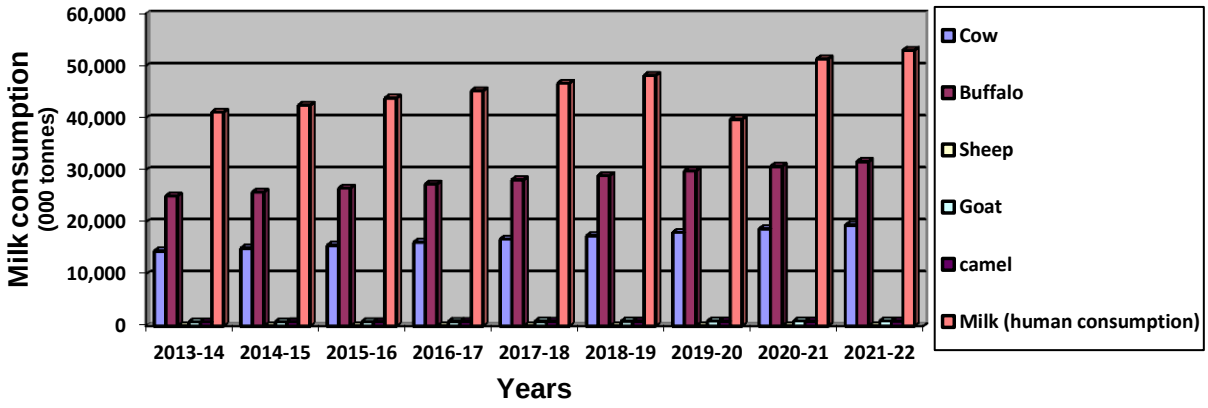

Figure 3: A graphical illustration of estimated human milk consumption in the years 2013-22
Source: Pakistan Economic Survey

5. Livestock Population

Table 3 demonstrates an increase in cattle and buffalo farming, with respective shares rising to 53.4% and 43.7%, respectively. Goat farming increased dramatically to 82.5%, while sheep farming stayed steady at 30%. Figure 4 provides additional information on the increased number of cattle between 2013 and 2022.

Table 3: Cattle population (2013-2022) in percentage

Species	2013-14	2014-15	2015-16	2016-17	2017-18	2018-19	2019-20	2020-21	2021-22
Cattle	39.7	41.2	42.8	44.4	46.1	47.8	49.6	51.5	53.4
Buffalo	34.6	35.6	36.6	37.7	38.8	40.0	41.2	42.4	43.7
Sheep	29.1	29.4	29.8	30.1	30.5	30.9	31.2	31.6	31.9
Goat	66.6	68.4	70.3	72.2	74.1	76.1	78.2	80.3	82.5

Source: Pakistan Economic Survey (2013-22). This statistic is based on the inter-census rate of growth of livestock censuses conducted in 1996 and 2006.

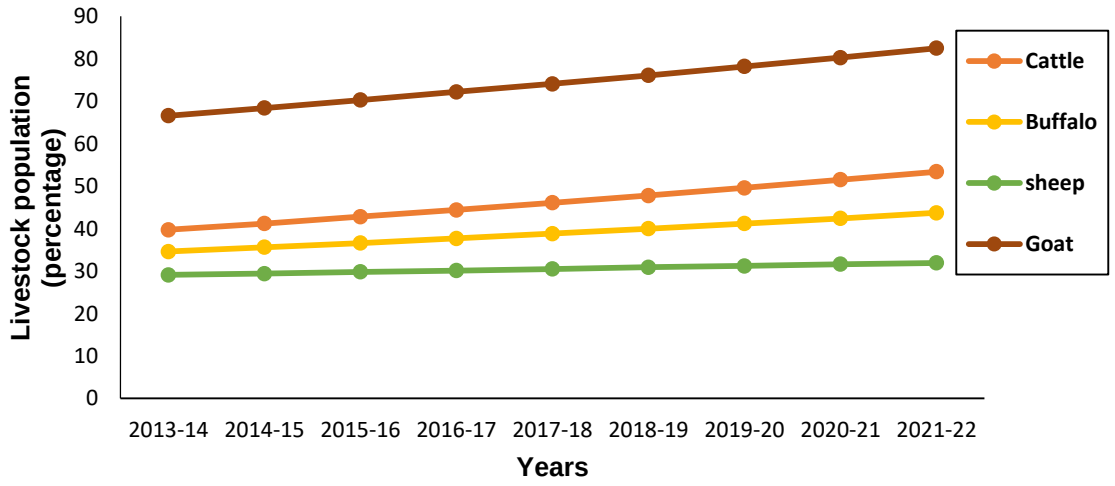

Figure 4: A graphical representation of the Livestock population from 2013 to 2022

6. Issues Regarding the Dairy Industry

Pakistan's dairy sector ranks fifth globally, generating a production volume of $26 billion across urban and rural areas [14]. Despite a growing population and rising domestic consumption, the milk output exceeds the population's demands. The daily shortage of milk in Karachi alone is approximately four million liters, and the demand and supply gap is projected to reach 3.6 billion liters by 2015 [15]. The rationale behind this phenomenon is rooted in the fact that the yearly rise in milk production cannot match the concurrent 3% expansion in both per capita consumption and human population. While milk production has indeed seen an uptick over time, this growth is attributed not to enhanced productivity per individual animal but rather to a surge in the overall count of animals [16]. The dairy industry's low productivity can be attributed to several factors, such as inadequate genetic resources, delayed puberty, suboptimal feed availability, high incidence of diseases, absence of a well-structured marketing system for livestock, inadequate research facilities, shortage of veterinarians, and insufficient rural infrastructure [17]. The COVID-19 pandemic brought significant distraction to the global dairy economy. The milk demand experienced daily escalation; however, the supply contracted amid the pandemic. Consequently, a substantial disparity emerged between market demand and the potential supply chain [18]. The devastating figure of 57.3 billion liters of unsold milk alongside other dairy products due to COVID-19 highlights the need for an irrepressible food system and sustenance for farmers [19].

Pakistan ranks third globally in animal herd size, with roughly 63 million animals, per the 2009 Economic Survey of Pakistan [20]. However, small-holding dairy farmers face a significant challenge in milk productivity due to the need for genetic resources and other factors [21]. During the lactation cycle, the milk productivity of cattle and buffalos does not exceed 4-5 liters per day. In Pakistan, it takes about 8 milk-producing animals to equal the productivity of a single animal in the developed world [22]. Additionally, milk production is affected by seasonal changes, with a 55% drop in peak production during May and June, which coincides with a 60% increase in demand compared to December, when there is abundant supply [23]. Milk's quality and shelf life are also compromised during May, yet the prices increase due to market shortages [24].

Previously, animals were smuggled or imported from Afghanistan, but the livestock sector has been adversely affected by the war in Afghanistan since 9/11[25]. Pakistan increased its livestock exports to Afghanistan, Iran, and Gulf states as a result of mad cow disease in Europe, which created a demand for livestock in these regions. This led to a shortage of raw materials for leather industries, prompting the government to take corrective measures to ensure a steady supply. Despite the potential for growth in Pakistan's Dairy Sector, various loopholes hinder its development. There needs to be more cooperation, and a clear plan for dairy progress among governing bodies and supporting organizations is a contributing factor [26]. Adulteration and unhygienic practices during milk handling by intermediaries, who add ice and sometimes vegetable oil to the milk, pose health risks [27]. Although government regulation could address this issue, the lack of developed milk collection systems means that only a tiny portion of the milk is appropriately collected and handled [28].

6.1. Insufficient supply of nutrients

Enhancing the quality and quantity of feed can increase livestock productivity by up to 50% [29]. In Pakistan, crops, bushes, grasses, and waste meet dairy animals' nutritional needs [30]. Foraging provides up to 95% of the nutrients [31], but the lack of available feed and fodder limits milk production [32]. The shrinking area for fodder production and its short supply time worsen the situation [33]. Pakistan necessitates around 10.9 of crude protein (CP) and 90.36 million tons of total digestible nutrients (TDN) per year for its 121.1 million livestock. According to AKRAM and FIRINCIOLU (2019), Livestock currently receives 51% of their nutritional needs from green fodder, 38% from crop wastes,3% from grazing vacant land, 6 % from post-harvest grazing, and 2% from cereal by-products, and oilcake and meals, respectively. The nutrient gaping hole could be reduced with better agricultural practices and inputs.

6.2. Decline of range lands

Ranges are vital in resource management, with rangelands encompassing 63% of Pakistan's total land area [34]. Rangelands are projected to provide 38% of Pakistan's livestock feed, establishing the second most significant contribution, surpassed only by

remnants of fodder-crop cultivation (51%) [35]. The poor pasture quality of these rangelands is one of the focal reasons for the decreased productivity of our animals [36]. Rangelands are undergoing degradation through overgrazing, nutrient deficiencies, rapid deforestation, and the uprooting of their range of vegetation. The deterioration of rangelands is evident, while effective strategies to sustain their productivity are still lacking. Historically, minimal research endeavors have been dedicated to enhancing rangeland conditions.

6.3. Animal health issues

In 2019–20, Pakistan had about 201 million livestock, primarily buffaloes and cows, at risk of diseases due to factors like contaminated water, improper feeding, and harsh weather [37]. Farmers do not fully utilize their benefits despite the availability of veterinary clinics, with only about 10% of the cattle herd receiving annual vaccinations [38]. A continuous vaccination program for diseases like foot and mouth is lacking, significantly impacting production. Major endemic livestock diseases in Pakistan include Foot and Mouth Disease (FMD), Hemorrhagic Septicemia (HS), Black Quarter (BQ), and diseases like sheep pox, anthrax, and enterotoxemia in sheep and goats [39].

6.4. Marketing-related concerns

Milk marketing in Pakistan faces challenges like inadequate infrastructure, leading to spoilage and waste. Lack of refrigeration, transport, and processing facilities causes up to 20% of milk wastage [40, 41]. The system disadvantages small producers, with middlemen monopolizing the market [42]. The scarcity of collection centers and limited testing facilities result in the sale of substandard milk [43, 44]. Seasonal production fluctuations, storage issues, and adulteration practices exacerbate the problem [45].

6.5. A lack of value-added resources

Within this nation, a scarcity of value-added infrastructure is glaringly evident [46]. The importance of this perilous issue must be given top precedence if dairying is to become a more lucrative sector. Value-added animal goods and byproducts are in high

demand and sold at high rates in the global markets. Flavors and colors are designed to influence consumers' preferences while considering their taste profile eating habits. Value-added milk from goats and camels is in high demand worldwide, and this industry has the potential to increase profitability.

6.6. Issue of peri-urban dairy colonies

Peri-urban dairy colonies in Pakistan face numerous challenges that impact the dairy industry and the local communities. Rapid urbanization leads to competition for agricultural land, reducing grazing areas for dairy farming [47]. Environmental pollution from animal waste affects water sources and poses health risks [48]. Inadequate infrastructure, including roads and sanitation facilities, hampers transportation and market access. The Proximity of humans and animals raises sanitation and disease concerns [49]. Limited veterinary services result in higher livestock mortality rates and reduced productivity. Market linkages and value chain integration are insufficient, hindering farmers' access to fair markets and value-added opportunities. Technical knowledge and training are lacking, impeding the adoption of modern farming practices.

6.7. Institutional limitations

Research institutes and experimental units face poor conditions due to inadequate employee training, insufficient budgets, and lack of farmer involvement [5]. Financial institutions provide limited support to small livestock farmers, leading to the need for microfinance. Livestock policies should focus on breeding animals to improve dairy productivity [50, 51]. Unfortunately, the country lacks facilities for training farmers in the latest technology, and technical training institutions don't provide quality information on livestock production problems.

6.8. The potential risks to breeds of livestock

Livestock diversity is declining due to environmental and societal factors [51]. In Pakistan, several potential threats, such as changing climate patterns, urbanization, diseases, inbreeding, loss of knowledge, and insufficient investment in breeding

programs and infrastructure, endanger the survival and genetic diversity of livestock breeds [52].

6.9. Limited services for the extension

Insufficient extension services in dairy production lead to low productivity due to farmers' reluctance to adopt modern technologies, limited investment, and high input costs [53, 54]. Proper extension services, efficient disease control programs, and good husbandry practices can enhance dairy animal production in remote areas [55]. However, extension personnel struggle to comprehend the actual needs of rural farmers, and programs that fail to enhance animal output are detrimental to livestock development [56].

6.10. Low production of dairy cows

The current animal productivity level is not enough to meet the rising demand for livestock products [57]. Pakistan lags behind other countries in terms of animal productivity per worker [58]. In Pakistan, it takes three dairy animals to produce the same amount of milk as one dairy animal in New Zealand. The reason for this difference in milk output is that the animal population is increasing at a faster rate than the resources required for livestock production.

6.11. Ineffective livestock services

The country's dairy animal and dairy farming departments are still operating at their original levels as veterinary services, offering limited health facilities and little animal production. The facilities are rendered by veterinarians with minimal education and skill sets and technicians with insufficient training [59]. They offer clinic-based services, but the extension staff has limited mobility. Consequently, less than 25% of the population can access animal health treatments. Breeding services are also scarce, as seen by the coverage of artificial insemination services, estimated to be no more than 3%, neglecting rural or isolated locations. Due to the government's lack of incentives for the livestock industry, livestock services have performed worse over time.

6.12. Inadequate growth of the milk processing sector

The poor development of the dairy industry in Pakistan poses significant challenges and hampers the growth and potential of the dairy sector [60]. One major issue is the lack of modern and efficient milk processing plants. The existing organization is characterized by its outdated amenities, lacking the advanced technologies needed for large-scale processing and production of value-added dairy products. The dearth of funds and resources hamper the establishment of new processing plants and the modernization of existing facilities [61]. The lack of robust infrastructure, including inadequate cold storage, transportation systems, and storage facilities, and the absence of rigorous quality control and standardization procedures also contribute to the underdevelopment of the milk processing sector [62].

6.13. Lack of policy

The absence of a comprehensive and well-executed policy framework for the dairy sector in Pakistan acts as a significant blockade to its growth and development [63]. Pakistan has witnessed the disappearance of over 36% of its indigenous livestock breeds due to the lack of vital conservation efforts and supportive government policies, according to a Food and Agriculture Organization (FAO) report. This alarming statistic highlights the pressing need for targeted policies to foster breed improvement and conserve genetic resources. The Pakistan Dairy Development Company reports that a significant amount of 85-90% of milk produced in the country remains raw and unprocessed [40], indicating the need for policy interpolations to boost processing ability. The absence of robust quality control and food safety regulations is also concern in the Pakistan dairy sector. A study led by the Pakistan Council for Scientific and Industrial Research (PCSIR) found that approximately 70% of sampled milk collected from different cities in Pakistan did not meet quality standards.

6.14. Breeds and breeding issues

Strategic breeding practices and breeding issues in the livestock sector play a pivotal role in the development and long-term sustainability of Pakistan's agricultural economy [64]. However, Livestock breed management in Pakistan faces challenges like genetic

erosion, loss of diversity, and inbreeding, leading to reduced productivity and disease susceptibility. The lack of farmer awareness and established breeding programs exacerbates these issues.

6.15. Limited access to Credit and Finance

The underperformance of research institutes can be attributed to various factors, including inadequate funding, underqualified staff, and low farmer involvement [65]. These institutions frequently provide technological packages that are incompatible with agricultural methods. Little loan support is available for small livestock farmers; most of it goes to influential people. Microfinance services and policies prioritizing animal breeding are necessary [66, 67]. The situation is particularly alarming in Baluchistan province, where no institution with the necessary capabilities can tackle these issues effectively. There are insufficient training facilities for technicians and farmers, especially regarding husbandry, feeding, and breeding.

6.16. Little understanding among small dairy farmers

Approximately 66% of rural population habitations depend on agriculture and animals [68]. They rely heavily on it as a primary source of income to meet their basic requirements. About 84 percent of small-scale dairy producers and their herds are in Pakistan's rural areas [69]. Britt, J. et al. state that 2.5 million dairy farms with a larger herd size can use current dairy equipment. However, most small dairy farmers lack education, are dogmatic, struggle to make ends meet, and are hesitant to adopt modern dairy practices because they are unaware of them [47].

6.17. Health and safety issues

Unsanitary production facilities and adulteration practices often compromise milk quality. Traditionally, boiling milk has been used to ensure safety for consumption. Poor hygiene, adulteration, and a broken cold chain produce low-quality milk. Public interest in milk supply chain safety has grown, making it a prominent issue in the dairy industry. It's crucial to examine safety failure factors in the dairy supply chain due to their direct impact on human consumption.

6.18. Dairy animals with great genetic potential as replacements

Replacing dairy heifers is vital for commercial dairy farming. Large farms often import seed stock from technologically advanced nations due to inadequate local breeding practices [70]. Replacing old genetic stock with new, high-milk-producing varieties is essential to maintain profitability. Importing livestock is costly and unsustainable, making local cultivation of replacement stock necessary. However, locally producing cost-effective, high-genetic-potential bovine heifers is challenging and requires significant research [71].

6.19. Factors to take into account with β-casein proteins

Casein, making up 80% of milk's protein, has various forms, with β-casein being the most common [72, 73]. The two main variants, A1 and A2, differ in their health impacts. A1, found in high quantities in Northern European breeds, releases the opioid-like B-casomorphin7 (BCM7) during digestion, potentially negatively affecting the body's neurological, endocrine, and immunological systems [74]. Some link A1 to health risks like autism, schizophrenia, type 1 diabetes, and coronary heart disease, though this is disputed [75]. The A1 and A2 milk controversy necessitates more precise research, including studies on various animal species. If A1-casein is proven harmless, it could significantly alter dairy farming practices globally.

6.20. Existence of antibiotics and Aflatoxins

6.20.1. Antibiotics

Antibiotic contamination in milk is a global subject, particularly in nations like Pakistan, where hygiene standards are low. Antibiotic residues refer to high levels of antibiotics or their derivatives in animal meat or byproducts. These residues pose direct health risks to humans, contaminate environmental resources, and interfere with dairy production [76]. International organizations, including the FAO, WHO, CAC, and EEC, aim to reduce antibiotic residues in milk and establish the standard maximum residual levels (MRLS) for animals and their products.

6.20.2. Mycotoxins

Aflatoxins, produced by Aspergillus species of fungi, are the most common mycotoxins found in various foods and feeds. These toxins are a global issue, especially in temperate regions where conditions favor fungal growth [77]. More than 20 different derivatives of aflatoxins exist, with acute exposure leading to high fatality rates [78]. However, most infections occur due to chronic exposure, affecting 4.5 billion people annually by weakening their immune systems [79]. The primary metabolite of aflatoxins in milk is aflatoxin M1, associated with severe health issues.

6.21. Factors to consider when comparing operating costs

Modern dairy operations involve high costs due to factors like elite livestock, feed ingredients, preventive medication, and electricity. These costs vary across milk production technologies and directly impact product pricing and profit margins [80]. Therefore, farmers must produce milk at lower costs to make Pakistan's dairy industry globally competitive. A pricing system per kg of milk production is also recommended, considering variations in species and milk production systems.

7. Proposed Strategies to Overcome Obstacles in the Dairy Sector

7.1. Adding value to dairy

Value-added dairy products provide benefits beyond essential nutrition. They can be flavored, fortified, or uniquely packaged. These products enhance the dairy industry's financial sustainability and competitiveness, opening new market opportunities and appealing to discerning consumers willing to pay a premium for tailored products [81].

7.2. Promoting the use of technology

Promoting technology in dairy farming increases efficiency and profitability. Precision farming uses sensors and data for optimal management. It monitors cow health milk production and identifies health issues early [82]. Automated milking systems reduce labor costs and improve efficiency [83]. Mobile apps provide real-time information on milk prices, market demand, and weather [84].

7.3. Modernizing supply chain management for success

Modern supply chain management is a great idea to encourage sustainable dairy practices [85]. Modern supply chain management can help minimize the environmental effects of dairy production by optimizing the manufacturing, handling, and transportation of milk and milk-derived products. In Figure 5, there is a proposed dairy supply chain model.

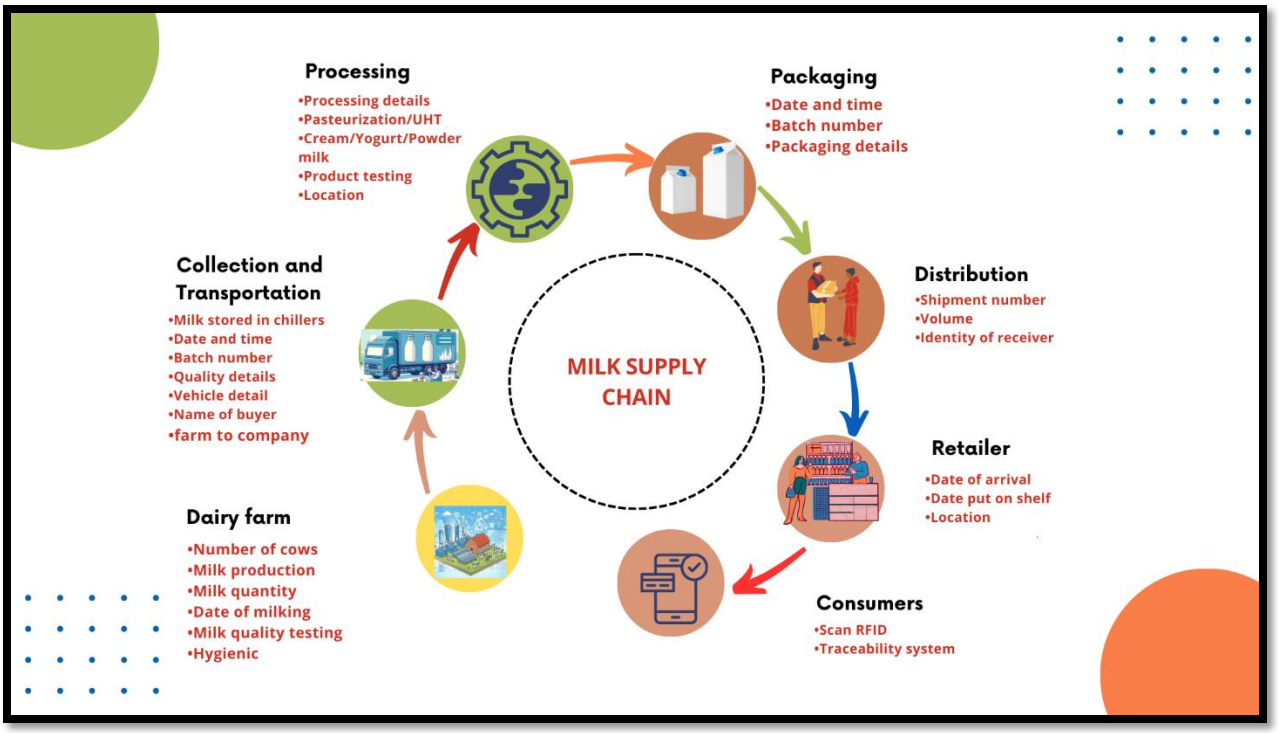

Figure 5: A proposed dairy supply chain model

7.4. Enhance range management

Effective dairy farming range management can be achieved through rotational grazing, infrastructure development, soil testing, reseeding, and monitoring [86]. Weed and pest control, expert partnerships, and community engagement are also crucial. Rangeland restoration and education further enhance sustainability, improving dairy production and environmental impact [87].

7.5. Improving animal health services

Enhancing animal health services is vital for Pakistan's dairy sector. This involves better veterinary care, vaccinations, disease prevention, regular health check-ups, and prompt treatment. Training veterinary professionals and public-private partnerships can improve service availability and affordability [88].

7.6. Improve market access

Enhancing market access is key for Pakistan's dairy farming. This includes infrastructure upgrades, stronger market connections, access to market data, policy engagement, and value addition. Better transport and cold chain facilities allow efficient product delivery. Stronger market links, accurate market data, and policy engagement help farmers secure better prices and access larger markets [89, 90].

7.7. Address peri-urban dairy challenges

Efficient strategies to tackle peri-urban dairy challenges in Pakistan include zoning regulations, proper waste management, improved herd management, farmer training, and collaboration with local authorities. These measures foster economic growth, ensure sustainable dairy farming, and enhance animal welfare and productivity [91].

7.8. Strengthen institutional support

Institutional support is crucial for Pakistan's dairy farming progress [92]. Farmers depend on organizations for advice, resources, and services, which enhance their knowledge and farming techniques. Access to modern technologies improves breeding, nutrition, and disease control. Farmer cooperatives facilitate group buying, marketing, and resource sharing. Enhanced institutional aid empowers farmers, increasing production, profits, and sustainability.

7.9. Protect livestock genetic diversity

Preserving livestock genetic diversity is essential for sustainable dairy farming. Artificial insemination (AI) is a vital tool in this, allowing controlled breeding with superior sires, maintaining genetic diversity, and preventing inbreeding [93, 94]. AI provides access to

high-quality genetics, improving the quality and productivity of dairy herds. By using AI responsibly and with proper selection criteria, farmers can preserve desirable traits and genetic diversity, ensuring long-term success in the dairy industry.

7.10. Foster milk industry

Fostering the milk processing industry requires creating modern processing plants, building solid relationships between producers and processors, investing in research and innovation, and receiving government assistance. These initiatives support the manufacturing of superior milk and dairy products, adapt to shifting consumer demands, and advance the economy.

7.11. Develop livestock guidelines

To achieve sustainable growth in Pakistan's livestock sector, it is essential to implement comprehensive policies, involve stakeholders, promote innovation, establish regulatory frameworks, and provide support to farmers [95]. The existing National Livestock Policy and Livestock and Dairy Development Policy have already contributed to increased milk production and the development of efficient milk collection and chilling systems. Further government initiatives are necessary to enhance the livestock industry further, which will ultimately improve Pakistan's food security and economic progress.

8. Conclusion

In summary, this chapter examined Pakistan's dairy industry, which comprises small holdings that engage in various farming practices. The dairy industry is operating sustainably to fulfill the needs of the expanding population regarding food. However, issues still need to be resolved for the dairy business soon, including local alternatives for dairy animals with high genetic traits, potential health concerns from B-casein proteins, antibiotics, and Aflatoxins consumption, and high operational costs. Gaining insight into the existing state of affairs and all possible solutions covered in this paper could facilitate addressing particular problems to overcome these obstacles.

Author Contributions

Conceptualization, F.S.; validation, F.S and M.A; writing—original draft preparation, Z.A, K.M, and A.G.; writing—review and editing, F.S, M.A and K.M; visualization, Z.A and A.G.

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Conflicts of Interest

The authors declare no conflict of interest.

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DAIRY SCIENCES - CURRENT TECHNOLOGICAL TRENDS AND FUTURE PERSPECTIVES

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REVIEW BASED BOOK CHAPTER

Application of Innovative Technologies in Fermented Dairy Products

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Abstract

Fermentation of dairy products is a way of preserving the nutrients found in milk and this fermentation is performed by diverse microbiota. Probiotic products (yogurt, cheese, and kefir) have been considered beneficial for human health. Their demands have reached a great length by consumers due to their high nutritional value and their enticing sensory characteristics worldwide. Different innovative processing technologies (Non-Thermal Technologies) are in use to further enhance their sensory characteristics such as flavor, texture, and preservation. The insights provided by these studies are summarized in this chapter.

Keywords

Fermentation, Cheese, Kefir, Yogurt, Innovative Methods

1. Introduction

Conventionally, fermentation is a process that is used for the preservation of food products and also ensures food safety. It boosts the nutritional, health-improving qualities of food and also increases its organoleptic characteristics. Many microbes can be used to ferment different dairy products [1]. Lactic acid bacteria (LAB) synthesize exopolysaccharides (EPS) that play a crucial role in the processing of dairy products [2]. A lactic acid bacterium, lactobacillus helveticus is a non-spore-forming gram-positive bacteria, which shows thermophilic, and homo fermentative abilities. This bacterium grows at 42°C temperature, which is specifically safe for the fermentation of dairy products after its isolation from dairy products [3-6].

Fermented dairy products are considered important ingredients in functional foods. Incorporation of probiotics to form high-value products has gained great interest recently. Different dairy products are greatly consumed such as yogurt, cheese, and kefir, etc. Here we will discuss the history, processing, therapeutic potential, value addition, and new innovative technologies used in producing these products [7].

2. Cheese

In the past, cheese was manufactured by Egypt and Summer's people and after that, cheese manufacturing was performed in Rome also. The Middle East and Europe increased the production of cheese and later Oceania, North and South America produced 1000 different varieties of cheese. In the mid-nineteenth century, cheese was produced only at the domestic level but with time new highly developed technologies produced several principal varieties of cheese. About 35% of all milk is specifically used to manufacture cheese. Recently south and North America, Oceania, and Europe have manufactured 19 tons annually [8]. The distinctive nature of cheese results from two important components of milk: its characteristics and composition [9].

2.1. Therapeutic Potential of Cheese

Cheese is one of the healthiest foods in the world, which is available in various shapes, tastes, and textures with a significant nutritional value. Globally, more than 2000 types of cheese are available in specific textures, forms and tastes [10, 11]. Cheddar cheese can be advantageous for those who are hypolactic because it contains a significant level of calcium and negligible amount of lactose [12].

Making cheese involves dehydrating milk and adding additional preservatives including culture, acidity, salting, packaging, and storage. In a procedure known as syneresis, the rennet-induced milk coagulum is chopped and heated to remove moisture. The curds are then packed into fresh cheese after being rinsed and seasoned. For most cheese kinds, the pH gradually decreases to a value between 4.6 and 6.0 during the process [13].

In earlier times, cheese was developed biotechnologically and emerged as a

functional dairy product worldwide. Cheese comprises bioactive compounds such as polysaccharides, fatty acids, and peptides. These substances have several functional attributes such as anti-thrombotic, antitumor, anti-obesity, antioxidant, and anti-hypo-glycemic reactions, and also act as a mineral-binding agent. Lactic acid bacteria also act as probiotics, which is very helpful in increasing the good digestion and absorption of nutrients to improve gut health [14, 15].

2.2. Value Addition in Cheese

Value addition/fortification is a method to improve the nutritional value of food products. Cheese is fortified by the addition of essential micro-nutrients (vitamins, carotenoids, polyphenols, and polyunsaturated fatty acids), the addition of minerals (selenium, zinc, and iron), and also fortified by agrifood-industry by-products. Allogenic microbes that are from entomological origin can be used to increase the technological, organoleptic, and nutritional value of cheese [16]. In the earliest days of calving, mammary glands of mammals secrete milk, which consists of important substances such as minerals, vitamins, lipids, and proteins. This initial milk contains a good level of lysozymes, oligosaccharides, and lactoferrin substances with anti-oxidant, and anti-inflammatory qualities, and also strengthens the immune system of humans. These are also present in mature milk but in limited amounts [17, 18]. A starter culture of lactic acid bacteria, essential oils, and extracts of plants are used in the processing of cheese which is conveniently found and applied directly in milk and curd to make cheese with higher nutritional value [19, 20].

Colostrum is used as a functional ingredient to develop novel FPC (probiotic cream cottage cheese). For this purpose, *Acidophilus-bifidus thermophilus* culture and BCP (bovine colostrum powder used 1, 2, and 3 percent. Then the antioxidant properties, antibacterial activity, fatty acid profile, and physicochemical properties of food products were analyzed. By increasing the amount of colostrum powder in all samples of cheese during its manufacturing, antioxidant activity was increased by ($P < 0.05$) and the storage time of cheese also increased. 2% bovine colostrum powder is used for the fortification of cheese and it shows a higher value of benefits than other treatments. So,

the result is cheese which contains 2% of bovine colostrum powder shows greater health and nutritional effects [21].

The quality of food products has been greatly improved with advancement in the field of biotechnology and the microbial strains produced by biotechnological approaches also proved to be helpful in the designing of cheese with enhanced nutritional value. Bioactive peptides are released due to the hydrolysis of enzymes, which occur during process of fermentation & digestion of dairy [14, 15].

3. Yogurt

Yogurt is an industrial food product that has been used around for ages. Numerous studies have been conducted since yogurt's discovery in an attempt to understand better and enhance the taste and texture to benefit human health. Commercially, yogurt and other dairy food products are thought to have originated from the Middle East [22]. Initially, yogurt was synthesized at a small level without any standardized methods till the twentieth century. But after the twentieth century, it became more commercial and its production started at a high level [23].

Yogurt is derived from the bacterial fermentation of milk by two species of bacterial cultures, *Streptococcus thermophilus*, and *Lactobacillus bulgaricus* [24]. Along with these two bacteria, other species of bacteria are also present in yogurt. The presence of these additives in fermented food products either enhances their probiotic nature (living microbes) or taste and texture [25]. During the manufacturing of yogurt, lineal strains of *lactobacillus bulgaricus* genetically produce acetaldehyde in dairy products [26]. Lactic acid bacteria are one of the major constituents of yogurt because they are used and transform sugars into lactic acid. These lactic acid bacteria are also helpful in the reduction of harmful substances and in the prolonging of the shelf life of dairy food products [25].

3.1. Therapeutic Potential of Yogurt

Globally, the demand for yogurt is increasing tremendously by consumers after knowing its therapeutic properties and its great nutritional value. These beneficial effects include

the strengthening of the immune system, increasing HDL cholesterol levels, and controlling blood sugar levels, also used in the treatment of gut-related diseases like constipation, inflammation of the stomach, and diarrhea. Additionally, it prevents various death-taking diseases like colorectal cancer and dangerous bacterial infections [27-30].

3.2. Value Addition in Yogurt

To increase health benefits and nutrient contents of foods, vegetables, and fruits are used to add value to food products because they are rich in vitamins, organic compounds, polyphenols, and dietary fiber. Clinically pulp of Annona species, oleracea, carrots, and strawberry juices are added to yogurt for the improvement of flavor and nutritional status [31-35]. Especially, for the enrichment of fermented dairy products such as yogurt, fruit juices are the best options. For this purpose, passion fruits are considered suitable because they are rich in many different organic compounds that inhibit the proliferation of infectious bacteria by decreasing the pH of fermented foods [36].

Malnutrition is a major problem all over the world, to overcome this problem food is enriched with essential nutrients. Due to the high consumption of yogurt, it is enriched with micronutrients like vitamin D_3, B_9, and B_7, minerals, bioactive compounds, and dietary fibers [37]. A Fortified puree of pineapple and coconut milk is used for the enrichment of yogurt because pineapple is rich in (bromelain enzymes, antioxidants, phenols, and organic substances) that lower the fat content in yogurt, and the addition of coconut milk (enriched in phenols, organic compounds, and proteins) which are found to be beneficial for human health [38].

4. Kefir

Kefir is an acidic and less alcoholic drink produced by the fermentation of milk or glucose water with kefir grains. Its origins may be traced to the Caucasus, Eastern Europe, and the Balkans. Because of its health-promoting qualities, its use has spread throughout the world throughout time. People in the US, Japan, France, Brazil, and

other nations have grown to love this tangy, viscous beverage [39-42]. Kefir word is derived from the Turkish language "kef" which means delight sensation. The people of the Middle East use this word commonly [43].

Chemically, kefir consists of different kinds of yeast and bacteria and is helpful in the enhancement of its sensory attributes [44]. Different kinds of drinks and foods are synthesized by using kefir grain to add additional characteristics like pleasant smell and taste to the final product [45]. Kefir grains are considered to be a hub of beneficial microorganisms. These grains consist of about thirty varieties of lactic acid bacteria that include Lactotococcus, Lactobacillus, leuconostic species and yeast includes *Torsulaspora, Saccharomyces, Candida, Kluyveromyces, Mycotorula* species [46].

These microbes develop, spread, and impart their distinctive traits to the subsequent generations of grain. There exists a mutualistic symbiotic relationship between these microorganisms and are also present in an inactivated form inside of a protein-polysaccharides pattern [47-49]. These grains of kefir are considered irregular, lightly yellow, or gelatinous lumps and also have an elastic texture. They vary in size and range from 0.3-3.5 cm in diameter. Chemically, kefir grains consist of about water (83%), proteins (4-5%), and kefiran (9-10%) [50]. Researchers suggested that excellent quality of kefir is obtained by incubation of homogenized milk with 3 percent (V/V)) bacteria (*streptococcus* and *lactococci* species) and yeast (*streptococcus* and *lactococci* species) at 258 degrees temperature for 24 hours [51].

4.1. Therapeutic Potential of Kefir

Kefir shows exceptional therapeutic potential because of the presence of bioactive substances such as carbon dioxides, hydrogen peroxides, organic acids, ethanol, and exopolysaccharides and antibiotics like bacteriocins work together to enhance the health benefits of kefir [49, 52]. Kefir also provides a healing effect against several diseases such as diabetes, cancer, high blood pressure, and high cholesterol levels which cause heart attacks, different allergies, microbial disorders, and other inflammatory diseases [53-55].

4.2. Value Addition in Kefir

Kefir presents an advanced level of physiochemical and biological properties, which is why different nutritious food additives and juices are added to increase its nutritional value. Recently, many researchers have worked on fortification and enrichment of kefir, to increase shelf life, health benefits, and nutrient contents. Encapsulated blackberry juice is considered a successful additive for the enrichment of kefir because of its exceptional morphological, physiochemical, and microbial safety behavior [56]. Recent research shows that the addition of hazelnut milk in kefir dairy products surprisingly increased the nutrient contents, shelf life, antioxidant potential, and phenolic substances in the final product. It is also observed that after fortification of kefir beverage organic and bioactive properties also improved [57]. Da Costa studied that People who are allergic to dairy foods can drink a kefir beverage, which is a combination of fermented extracts of beans, sesame seeds, and yam [58].

5. Innovative Technologies

New innovative methods are applied for the fermentation of dairy products. Recently, non-thermal technologies have been applied in the dairy industry other than thermal technologies because of their high potential for properties in fermented dairy products.

Non-thermal technology involves the processing of food in a limited time while keeping the temperature lower. These conditions ensure the safety of food and enhance the shelf life of dairy products. Non-thermal technologies (high pressure, ultrasound, and pulsed electric fields) provide high-quality dairy products with fresh-like characteristics [59, 60].

5.1. Ultrasound Technology

New technologies are developed to get safe, nutritious, energy-efficient, and cost-effective food products with a high acceptance level from consumers. Ultrasound technology proved to be one of the most effective technologies to meet the increasing demands of consumers [61, 62]. Ultrasound is waves that exhibit a frequency higher than the human hearing range (>18 kHz), produced by an instrument called

ultrasonic transducers, that convert electric energy to vibration form. Although the frequency of the spectrum of ultrasound waves ranges from 20kHz-1MHz, in food industries 20-40kHz frequency range is applied [63]. Ultrasounds are categorized into two types based on their frequency range.

i. High-frequency and Low-intensity

These waves frequency ranges at 100kHz and its intensity is less than 1Watt cm^2. These waves are also considered low-power and energy waves [64]. Soria and Villamiel studied low-frequency ultrasound waves used to check the acidic behavior, interaction of proteins, ripeness, and firmness of dairy products [65].

ii. Low-frequency and High-intensity

These ultrasound waves use a frequency that ranges from 20-500k Hz and its intensity is greater than 1W cm^2 [64]. Psiyasena, Mohareb and McKellar researched the applications of these waves on fermented dairy products and found that they are involved in the inactivation of microbes during fermentation because improving the viability of microbes is very important to get high-quality final product [66]. Soria and Villamiel, also proved that high-intensity ultrasound waves alternate the chemical and physical properties of dairy products [65].

Recently Galvan and his colleagues found that ultrasound technology is greatly used in the fermentation of dairy products because fermentation is a slow process that needs more time for the conversion of organic substances to simple form during the action of enzymes and microbe enzymes [67].

In fermented dairy industries ultrasound technology is applied to reduce the viscosity, for extraction of novel nutrients, increase ultrafiltration of whey protein, and is used in fat homogenization [68]. Pitt and Rose researched the effect of ultrasound on the cell growth of microbes by protecting the transfer of waste products and enhancing the transport of nutrient contents and oxygen to cells which proved to increase the viability of microbes by increasing their growth [69].

5.1.1. Application in Yogurt Processing

Sfakianakis and Tzia research that ultrasound waves in the production of yogurt are a new innovative method for better sensory and physiochemical characteristics of the final product. When ultrasound intensity increased and its exposure time on milk also increased, it was observed that yogurt showed greater viscosity and water-holding capacity [70]. Clinically milk treated with ultrasound treatment (25 kHz, 400 W, 45 or 75 °C for 10 min) produces high-quality yogurt. Processing innovations in the cheese industry have been proven to be of great importance in improving the functional attributes, flavors, and textures and also in prolonging the shelf life of food products [71].

5.1.2. Applications in Cheese Processing

In the cheese industry, ultrasound technology is used in the cutting of cheese. High-energy acoustic vibrations of ultrasound instruments do not damage the structure and shape of cheese, work efficiently, and reduce the loss of the final product [68, 72, 73]. High-intensity ultrasound is used to cut all types of cheese such as Swiss, mozzarella, and cheddar cheese. These high-intensity sound waves do not affect the quality of cheese during storage of cheese after it is cut for 21 days and stored at 4°C [72].

5.1.3. Applications in Kefir Processing

Düven, Kumcuoğlu and Kışla [74] researched the impact of ultrasound waves during the fermentation of kefir by applying 24kHz frequency, 22millimeter prob with 30 percent amplitude for 5 minutes and analyzed different parameters such as acidity, microbial activity, and pH. Their research results show that after the ultrasound application exopolysaccharides were produced and the time of fermentation for kefir production decreased by 1 hour and this technology positively improved the quality of kefir.

5.2. Pulsed Electric Field

In ancient times thermal processes were mostly used for the safety of food from harmful microbes but high temperature reduces the nutritional value of products by changing

their flavor, taste, and physiochemical properties that's why new technology which is pulsed electric field used recently to reduce the loss of nutrients and sensory characteristics of the final product [75, 76]. Application of different voltage pulses ranges from 1 to 40 kilo volt/centimeter for micro-milli seconds time in solid, liquid food products that are present between electrodes which produces an electric field, commercially used for the preservation of foods from harmful microbes is termed a pulsed electric field [77, 78].

5.2.1. Application in Cheese and Kefir Processing

Peng researched the effect of a pulsed electric field on the proliferation of microbes, and lactic acid bacteria (*Lactobacillus delbrueckii* subsp. *bulgaricus*) during the process of fermentation of dairy products like cheese and kefir. Different parameters such as pH, acidification rate, and microbial growth during the fermentation of dairy products are measured and analyzed. Different ranges of pulse from 60 to 428 V/cm or above were applied in microbial culture and observed their effects on these parameters. Peng and his colleagues observed that the application of electric field pulses sowed promising results reducing the pH, and acidity in and controlling the growth of microbes in the food product [79].

5.2.2. Application in Yogurt Processing

The inactivation of microbes is a very important factor in the manufacturing of yogurt, a new technique that uses pulsed electric field technology and produces high-quality final products. High-pressure electric pulses immobilized the cells of microbes, their cells burst and release their contents. Pulsed electric fields are applied to treat milk and inactivate the microbes. Milk is exposed to electric pulses in the presence of a probiotic culture, and the intensity of pulses ranges from 15-50 kV/cm for some seconds, finally, this process enhances the sensory characteristics of final products [70].

5.3. High-Pressure Technologies

This method is an advanced technological process to increase the shelf life of fermented products by applying pressure ranges from above 1000 M pascal. This

technology is not only used in preservation but also to maintain the nutrition of food without changing flavor, color, and aroma [80, 81]. High-pressure technology, high-pressure homogenization, and high hydrostatic pressure are all considered high-pressure treatments, and their effects on the dairy industry have been studied recently [82]. Serra and her colleagues studied that high-pressure homogenization technology increases the volatile acids (lactic acid and pyruvic acid), non-volatile acids (butyric and acetic acid), and these acids greatly increase aromatic compounds (diacetyl and acetaldehyde), and also sensory characteristics of the final product [83].

5.3.1. Application in Yogurt Processing

Mota studied that during the fermentation of milk for yogurt production high-pressure method greatly increases the viscosity of yogurt, denatures the whey proteins, and disturbs the casein micelles by reducing syneresis. When pressure between 100-300 M Pascals is applied in a starter culture, protein split into simple amino acids and peptides, which increases the growth of probiotics because pressure affects the metabolic process and activates stress response that improves the survival of probiotics during the process of fermentation [84].

5.3.2. Application in Cheese Processing

In the cheese industry, this technology helps to increase the process of salting, improve the texture also upgrade the coagulation of rennet and curd production [85]. Trujillo's [86] research shows that the application of High-pressure technology in cheese increases the number of amino acids, moisture, and salt contents in cheese made from pasteurized milk. Butz and his colleagues [87] & Iwanczak and Wisniewska [88] investigated that high hydrostatic pressure is also an innovative method that helps to control proteolysis during the ripening of cheese and also helps to reduce its maturation time. This technology is also applied to packaged cheese products because high pressure effectively controls the growth of pathogenic microorganisms like *Listeria monocytogenes.* When we apply pressure of 600 M pascals in fresh cheese, this pressure efficiently decreases the detection limit of bacteria (*Listeria monocytogenes)* which is 0.91 log colony forming unit per gram [89].

5.3.3. Application in Kefir Processing

According to Mainville [90], while some earlier research has examined the effect of high hydrostatic pressure (HHP) technology in kefir processing, it has always been with a particular emphasis on the microbial activity and preservation of this fermented dairy product. To the best of our knowledge, nevertheless, there is no evidence of how HHP processing affects other aspects of kefir quality. While concerning the impact of HHP processing on additional kefir quality parameters, no information is currently available. This is a crucial consideration for the creation of innovative dairy products as well as safe, marketable goods with acceptable sensory attributes [91].

6. In-line and On-line Analysis of Fermented Dairy Products

6.1. By Using Focused Beam Reflectance Measurement

In fermented dairy products, particle size greatly influences the relationship of viscosity during processing and sensory properties of fat-free fermented milk products. Heck, Nöbel and Hinrichs [92] work on the analysis of particle size inline by using focused beam reflectance measurement to get actual-time data of fermented dairy products, especially fresh cheese. They do the comparison of in-line and off-line particle sizes of fermented dairy products (fresh cheese) and found that this analysis helps to control different parameters like consistency, product flow rate and pressure, and downstream processing used to improve the viscosity of product after fermentation in fresh cheese processing. Further studies work to use artificial intelligence (AI) to collect correct data of different parameters by using inline sensors during the process of fermentation which will help to improve the quality and texture of cheese.

6.2. By Using the Multiple Light Scattering Method

Ramezani [93] looks at the monitoring of milk fermentation using multiple light scattering. The studies were conducted on milk that had undergone fermentation at temperature ranges (36-44°C) and with starter concentrations ranging from 0.05% to 4.5% (w/w). Additionally, antibiotics were present in quantities of up to 100 µg/kg. Multiple light scattering was used to continually monitor the fermentation process, and

a pH meter, rheometer, and texture analyzer were used concurrently. Changes in pH, rheological parameters, and sample hardness were shown to be associated with the backscattering signal captured by several light scattering measurements during the fermentation process. The elevated starter culture concentration of about 4.5% (w/w) with incubation temperature (44°C) resulted in a gelation period of 120 minutes. The pH, texture, and rheological monitoring supported these findings. Even at low dosages (1.3 µg/kg), the impact of antibiotics on gel formation could be detected using backscattering spectra analysis. Overall, the findings demonstrated the benefits of employing multiple light scattering as a quality control instrument for real-time milk fermentation monitoring.

Conclusion

Fermentation of foods by the action of microbes plays an essential role in the production of high-quality, nutritious food products. Nowadays, the consumption of yogurt, cheese, and kefir has greatly increased and is considered a main part of their diets. These products show a significant therapeutic potential. Also, different scientific research suggested that different extracts of fruit or essential oils can be used as an additive to improve the nutritional value of fermented dairy products. In ancient times thermal processes were used to preserve food products but these processes disturb the texture, color, and flavor of dairy products, that's why non-thermal technologies (ultrasounds, pulsed electric field, and high-pressure) have been developed to improve both sensory and nutritional profile of fermented dairy products. However new research is needed to analyze the different parameters that are involved in the processing of controlled fermentation of dairy products by using in-line and on-line techniques like multiple light scattering methods or focused beam reflectance measurement. Based on the reviewed scientific research, it can be concluded that innovative processing technologies and other techniques can significantly improve the nutritional and sensory properties of fermented dairy products.

Author Contributions

All authors equally contributed for manuscript preparation and editing.

Conflicts of Interest

The authors declare no conflict of interest.

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DAIRY SCIENCES - CURRENT TECHNOLOGICAL TRENDS AND FUTURE PERSPECTIVES

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REVIEW BASED BOOK CHAPTER

Recent Innovations in Detection of Aflatoxins in Dairy and other Food Products

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Abstract

Aflatoxins are naturally occurring secondary metabolites produced by Aspergillus flavus and Aspergillus parasitic, among other molds. The world's food safety and trading economy, particularly in developing nations, are seriously threatened by aflatoxins, the most dangerous natural mycotoxin yet identified. It contaminates a variety of products, including cotton, groundnuts, maize, and chilies, and has a devastating impact on both human and animal health. As a result, the global economy has suffered billion-dollar losses. Aflatoxin B1, B2, G1, and G2 are the most common and deadly of the over eighteen distinct forms of aflatoxins that have been identified so far. One of the most important aspects of controlling aflatoxin contamination is early fungal infection diagnosis. Aflatoxin contamination in crops and food items is therefore determined using a variety of techniques, such as chromatographic techniques, spectroscopic techniques, and immunochemical techniques. This chapter summarizes the recent methods for the detection and analysis of different aflatoxins in dairy and other food products.

Keywords
Aflatoxins, Food Safety, Detection Methods, Analytical Techniques, Biosensors

1. Introduction

A major family of secondary metabolites is known as aflatoxins, which are all derivatives of the difuran-coumarin and are mostly generated by Aspergillus flavus and Aspergillus parasiticus. While aflatoxins are nearly insoluble in water, they may be readily dissolved in a range of organic solvents including acetonitrile and methanol. The toxicity of food that has been tainted cannot be eliminated by cooking since they have a high thermal stability [1]. The most common naturally occurring toxins identified

in food and feed crops are aflatoxin B1 (AFB1), B2 (AFB2), G1 (AFG2), and G2 (AFG2), which make up all of the known kinds of AFs. Furthermore, milk and milk products include the hydroxylated forms of AFB1 and AFB2, respectively, which are formed when grazing animals eat infected crops. These compounds are known as aflatoxin M1 (AFM1) and M2 (AFM2) [2].

The most powerful and common aflatoxin is aflatoxin B1 (AFB1). The International Agency for Research on Cancer (IARC) has categorized it as a Group 1 carcinogen, suggesting that it is a substance that is known to cause cancer in humans [3]. The most potent and well-studied natural carcinogen, AFB1, is known to cause hepatocellular carcinoma, which is closely related to immunosuppression, malnutrition, and impaired growth [4]. Aflatoxin B2 (AFB2) is structurally identical to AFB1 and less frequently discovered in tainted foods. Another important aflatoxin that endangers the health of both people and animals is aflatoxin G1 (AFG1). It is created as a result of bacteria converting AFB1 via metabolism. Of the four main aflatoxins, aflatoxin G2 (AFG2) is the least prevalent. Additionally, it is produced through the metabolic conversion of AFB1 [3]. Aflatoxin M1 (AFM1) is a toxin that develops from aflatoxin B1 (AFB1) in breastfeeding animals through the hydroxylation reaction, which is performed by liver cytochrome P450. Raw milk from cows fed AFB1-contaminated feed will be AFM1-contaminated [5].

Aflatoxins have been proven to have a variety of negative consequences on health, including hepatotoxicity, mutagenesis, carcinogenesis, immunosuppression, and neurotoxicity effects on epigenetics, reproductive problems, and stunted development [6]. Vomiting, stomach discomfort, pulmonary edema, coma, fatty liver, kidney, and heart diseases are symptoms of acute aflatoxicosis in humans. AFs also have a synergistic impact with hepatitis B and C in humans by boosting a person's vulnerability ten times or more to liver cancer. According to estimates, 25% of the world's food crops are harmed by aflatoxin (AF) contamination, which has been cited as one of the biggest threats to food safety [7]. Due to the significant financial losses and negative effects on human health, food contamination with AFs is a concern on a global scale [8]. Because of this the majority of regulatory organizations for food safety implement

tight guidelines to reduce dietary exposure in people. There are several different forms of AFs, but only four naturally exist, including aflatoxin B1 (AFB1, AFB2, AFG1, and AFG2), which is known to be harmful to human health. The primary cause of AF food contamination at both the pre and post-harvest stages is fungal diseases. Climate factors that encourage fungal growth and AF formation include humidity, precipitation, and high temperatures. The presence of AFs in food is also influenced by poor storage and transportation practices. As AFs are heat-resistant so standard household cooking techniques cannot destroy those [9].

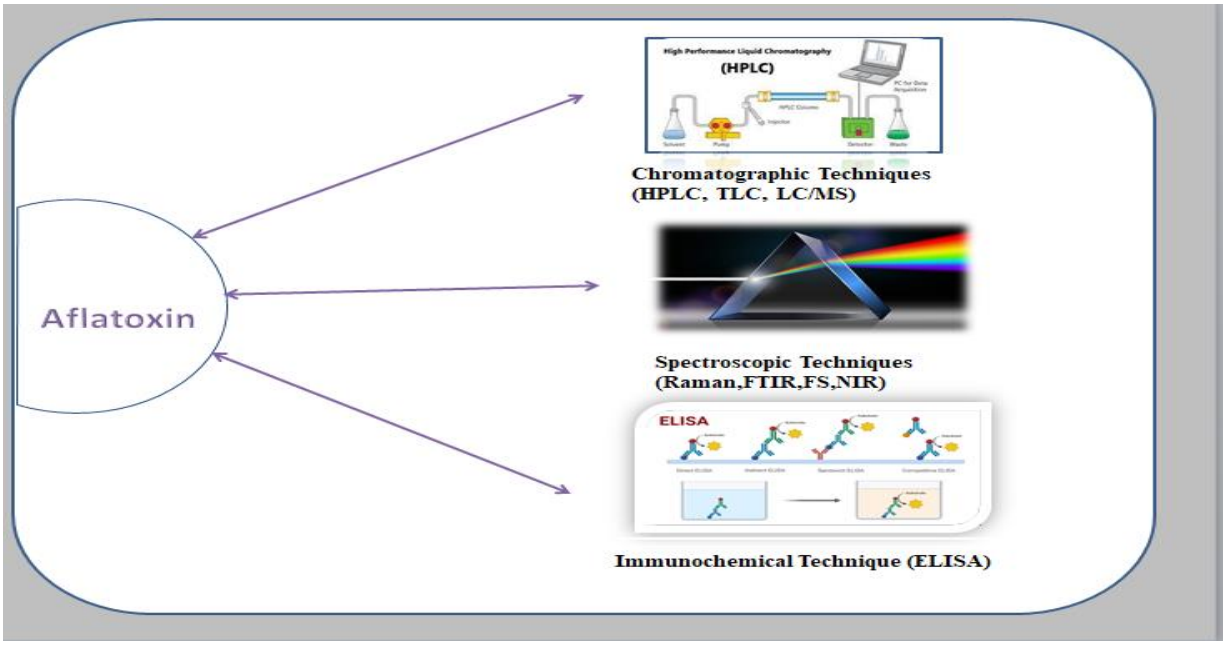

Figure 1 Detection of aflatoxin using chromatographic, spectroscopic and immunochemical techniques

2. Detection Techniques

2.1 Chromatographic Techniques

2.1.1 High-Performance Liquid Chromatography (HPLC)

A frequently used analytical technique for the separation, identification, and quantification of different chemicals, including aflatoxins, is high-performance liquid

chromatography (HPLC). The HPLC method makes use of liquid chromatography principles to separate the mixture's components [3].

Mycotoxins have been extensively analyzed over the past ten years using HPLC and other adsorbents. With either normal or reverse-phase HPLC, toxins have been separated based on their polarity [10]. Due to the composition of the milk or milk products, the procedures employed for HPLC detection of AFM1 may differ somewhat or need a different polarity of the mobile phase [11]. The majority of mycotoxins already contain natural fluorescence and may be identified using HPLC_FD, which is dependent on the existence of a chromophore in the molecules for fluorescence detection. The ability to integrate different detection methods allowing for the detection of many chemicals from a single component is the major benefit of employing HPLC, in addition to the high quality of separation and low LOD [12]. AFM1 was detected qualitatively and quantitatively in 130 samples of milk and some dairy products using the techniques of thin layer chromatography (TLC), high-performance]. liquid chromatography (HPLC) and enzyme-linked immunosorbent assay (ELISA). Additionally, the most significant amount of AFM1 in the local cheese was discovered with ELISA at 939.67ng/L and with HPLC at 300.7ng/L. Yogurt and cheese exhibited higher levels of AFM1 contamination than other items. The percentages of positive findings (contaminated with AFM1) were 38.5%, 50.5%, and 53.8%, in that order [13].

Because of its accuracy, sensitivity, and more precisely, the ability to identify aflatoxins as long as the concentration surpasses 0.015 ppb in ultra/high-performance liquid chromatography (UPLC/HPLC) has become an incredibly popular detection technology in recent years [1]. The variations in their physicochemical characteristics, such as polarity and hydrophobicity allow aflatoxins to be separated by HPLC. Usually, a reverse-phase HPLC column is employed, with the stationary phase being non-polar (hydrophobic) and the mobile phase being a polar solvent or their combination. Based on their respective polarity, this configuration enables the retention and extraction of aflatoxins [3]. A total of 40 samples were examined using the ELISA screening test and the confirmatory technique of high-performance liquid chromatography (HPLC) with fluorescence detection (FLD) following immunoaffinity column (IAC) clean-up. The

findings showed that there was no evidence of contamination in the butter samples from the Black Sea region with better qualitative characteristics in terms of AFM1, which proved as suitable for the detection of AFM1 in butter is the precise and sensitive IAC/HPLC-FLD technique [14]. 170 raw cow's milk samples from the Qazvin province's dairy farms, factories, milk collection facilities, and milk supply centers were gathered for this study during the winter months of 2013, and all samples were tested for AFM1. High-performance liquid chromatography and a commercially available competitive ELISA kit were used to evaluate the samples. All milk samples had AFM1 contamination. AFM1 contamination was found in 57 milk samples (33.52%), exceeding the threshold set by Iran's Institute of Standards and Industrial Research (0.5 ng/ml), whereas AFM1 content was within the allowable limits in 113 milk samples (66.481%) [15]. To identify AFM1 in milk and cheese, an HPLC technique with fluorescence detection was used after post-column derivatization with pyridinium hydrobromide perbromide. The average recoveries were 90% and 76%, respectively, for milk and cheese, which had detection limits of 0.001 g/kg and 0.005 g/kg, respectively. Additionally, the accuracy (RSDr) for cheese varied from 3.5% to 6.5% and for milk, it was from 1.7% to 2.6%. The experiment showed that the procedure was straightforward and amenable to automation, making it suitable for the precise and accurate study of AFM1 in milk and cheese [16]. The detection of AFM1 in milk and milk-based yogurt and cheese products was accomplished in this study using highly accurate and sensitive HPLC-FLD techniques along with IAC clean-up. In terms of parameters like specificity, sensitivity, accuracy, and precision of the analytical techniques for determining the presence of AFM1 in milk, yoghurt, and cheese, the fluorescence detection approach employing IAC clean-up produced good results. The techniques were tested for three distinct matrices at concentrations of AFM1 in milk that were permitted by European law. AFM1, which was identified in the dairy samples by HPLC-FLD at levels above LOD, was verified by LC-MS-MS [17].

2.1.2 Thin Layer Chromatography (TLC)

Aflatoxins fluoresce under UV light (365nm wavelength), which separates them on the thin plate due to their distinct adsorption capacities [1]. Thin-layer chromatography

(TLC), in line with AOAC standards, has been a recognized and frequently used technique for AF analysis since the 1990s. It is frequently utilized in laboratories throughout the globe to provide quality control of different food items and to conduct qualitative analysis [18]. The process uses a stationary phase usually made of glass or plastic or cellulose, silica, or alumina immobilized on it. While moving through the solid stationary phase, the analyte is transported by the mobile phase, which consists of water and an organic solvent combination.

Different kinds of AFs have varying affinities for the stationary phase, which causes them to either firmly attach to it or stay in the mobile phase. These affinities are caused by variances in the structure of their molecules and connections to the stationary and mobile phases. Thin-layer chromatography can effectively and efficiently separate mycotoxins due to this distinct behavior [19]. Thin-layer chromatography has been widely used to determine the amount of AF in a variety of food and feed samples, with reported detection limits as low as 1–20 ppb [20]. TLC techniques were mostly used to determine the presence of AFB1 in maize samples in 1978. TLC technique utilization has been observed to fall with time, from 53% in 1989 to 7% in 2002. TLC techniques are still advised at the moment for the identification of AF in any plant material. TLC techniques are occasionally used for mycotoxin screening in natural herbal medicines when fungal analysis has a cheap cost of detection and requires less equipment, it is researched together with concurrent mycotoxin contamination [21].

Thin layer chromatography was the first chromatographic technique, and it is still used today to quickly test for specific mycotoxins using either instrumented densitometry or eye inspection. The implementation of reliable, quick, simple, and low-cost methods that in a single run can identify and measure many mycotoxins with high selectivity and sensitivity is the focus of current trends in mycotoxin analysis in food [22]. When aflatoxins were first recognized as food contamination, thin-layer chromatography was the most widely used chromatographic method. Since then, advances in chromatographic technology have kept pace with advancements in mycotoxin analysis [23]. Aflatoxin may be found via thin-layer chromatography, which is a quick and easy procedure. In a nutshell, each test sample's aflatoxin is extracted using

organic solvents, spread, and identified at a particular wavelength via chromatography (e.g., 365 nm) following a series of steps. Aflatoxin will produce fluorescence that could be utilized to estimate the amount of aflatoxin [24].

Thin-layer chromatography is regarded as the first chromatographic method and is useful for quick mycotoxin screening. Despite being a cheap procedure, the measurements cannot be regarded as sensitive and precise. Each mycotoxin's physical and chemical makeup determines the appropriate cleanup phase and the sample preparation step is also important [25]. There are several screening techniques based on TLC that are applicable to AFM1 in milk, but only a few laboratories employ them since they don't offer a sufficient quantification limit (LC). A study involving 14 laboratories from 11 different countries was initiated in 2004 by the Food and Agriculture Organization (FAO), the International Atomic Energy Agency (IAEA), the International Union of Pure and Applied Chemistry (IUPAC), and the Committee on Food Chemistry. The goal of the study was to create a technique that might integrate the purification of immune affinity to ascertain AFM1 in milk using these three organizations' respective food chemistry committees [26]. The separation, evaluation of purity, and identification of organic compounds may all be done using thin-layer chromatography, a fairly ancient technique. In reality, it represented one of the techniques used for separation in previous AF analyses that was most often utilized. The Association of Analytical Communities (AOAC) also recognized thin-layer chromatography as an approved technique and the preferred approach for identifying and quantifying AFs at concentrations as low as 1 mg/g in 1990 [12]. A combination of methanol, acetonitrile, and water makes up the mobile phase of thin-layer chromatography that transports the sample while moving along the stationary phase. Thin-layer chromatography is a highly sensitive test that needs skilled specialists to perform, yet it is beneficial to identify many forms of mycotoxins in one test [27].

2.1.3 Liquid Chromatography/Mass Spectroscopy (LC/MS OR LC/MS/MS)

The material is extracted using the LC system; after ionization, the parent ions and fragments are separated using the MS system's mass analyzer based on the mass charge ratio [1]. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) has

become a popular method for determining aflatoxins in a variety of food matrices, including which includes fruits, vegetables, grains, and products made from them, as well as Chinese herbal remedies [28]. Aflatoxin detection, both quantitative and qualitative, has been the subject of a few researches on tea samples. Furthermore, one of the biggest obstacles to overcome before performing an LC-MS/MS quantitative analysis on a sample is the reduction of matrix effect. For this reason, an effective, precise, easy-to-use, and affordable pretreatment method is crucial to the success of LC-MS in determining the presence of aflatoxin in tea. Our objective was the development of a simple and efficient LC-MS/MS approach for the identification of aflatoxins in fermented tea by utilizing a special pretreatment methodology to achieve low threshold detection, high sensitivity, and the reduction of matrix effects [29].

The detection of aflatoxins (AFB1, AFB2, AFG1, and AFG2) in maize has been improved and verified using an LC-MS/MS technique. The method most frequently employed to identify mycotoxins nowadays is liquid chromatography linked to tandem mass spectrometry or LC MS/MS. Due to its great selectivity and sensitivity, it may be administered without a clean-up step or derivatization [30]. The experimental work revealed the effectiveness of the LC-MS/MS approach for the precise measurement of mycotoxin binders in the presence of aflatoxins: efficient sample preparation and administration. When developing a technique for quantifying mycotoxins in raw materials with mycotoxin binders, it is advised to employ LC-MS/MS as it enables the simultaneous determination of numerous mycotoxins [31]. The fastest-growing approach for mycotoxin analysis has been LC-MS since the 1980s. The advantages of mycotoxin analysis using LC-MS have long been acknowledged and utilized. It is possible to determine numerous mycotoxins at once using the approach by using LC isolation and the targeted analyte mass to charge (m/z). Compared to the extrinsic qualities employed by LC-UV/FLD, that include visible and ultraviolet absorption of light or fluorescence emission, this intrinsic attribute enables a more precise determination by considering the molecular weight of the target analyte [18]. The interfaces between LC and MS detectors, the restricted ionization efficiency of MS detectors, and the weak and unstable response of many mycotoxins due to inadequate desolvation were the constraints that hampered the adoption of LC-MS in the 1980s [32].

At the moment, LC-MS is the industry standard for mycotoxin analysis due to these extremely desirable properties. To determine the presence of aflatoxin B1 and M1 in milk, fresh milk, and milk powder samples, a new, completely automated approach based on dual-column switching employing online turbulent flow chromatography and LC-MS/MS was implemented [33]. AFM1 analysis in dairy products has been made possible by the growth of many LC-MS or LC-MS/MS techniques following the advent of mass spectrometry (MS) and its connection with LC [16]. To determine any possible correlations between each mycotoxin, this study examined the levels of contamination and co-occurrence of many mycotoxins using AFB1-positive samples from three different Traditional Chinese medicinal material (TCM) matrices [34]. Three distinct TCM matrices Polygalae Radix (PR), Coicis Semen (CS), and Eupolyphaga Steleophaga (ES) represent different matrices of wood fiber, starches, and proteins, respectively, and their respective mycotoxin levels were examined using the aforementioned method. The results of this study were utilized to forecast the likelihood of mycotoxin contamination and serve as a benchmark for the safe monitoring of TCMs and the establishment of guidelines and limitations [35].

Table 1 Chromatographic techniques for detecting aflatoxins in food and food products

Detection Techniques	Food Products	Aflatoxin	Reference
1. HPLC	1. Milk 2. Yogurt 3. Cheese 4. Butter	AFM1 AFM1 AFM1 AFM1	[12, 13, 14]
2. TLC	1. Maize 2. Milk	AFB1 AFM1	[21, 26]
3. LC/MS	1. Maize 2. Milk 3. Fresh milk 4. Milk powders	AFB1, AFB2, AFG1, AFG2 AFM1	[30, 33]

The detection of aflatoxins is a common usage of tandem mass spectrometry in conjunction with liquid chromatography, or LC/MS/MS, a potent analytical method. Aflatoxin analysis that is extremely selective and sensitive is achieved by combining the mass spectrometry's sensitivity and specificity with liquid chromatography's separation capabilities using the LC/MS/MS approach [3].

2.2 Spectroscopic Techniques

2.2.1 Raman Spectroscopy

Usually producing a faint signal, inelastic light scattering causes molecular vibrations that are studied using Raman spectroscopy. However, the strong electromagnetic field produced by the stimulation of the localized surface plasmon resonance (LSPR) can greatly increase the Raman signals when a sample is placed close to or on rough and noble metals nano-substrates [36].

2.2.1.1 Raman Spectroscopy in different food products

Surface-enhanced Raman scattering (SERS) has become a potent analytical method for the sensitive and quick identification of a wide range of food pollutants, including pathogenic microorganisms, allergies, pesticides, and microplastics [37]. Research work involved the quick detection of three prevalent mycotoxin types observed on maize at the same time: ochratoxin A (OTA), zearalenone (ZEN), and aflatoxin B1 (AFB1). It was achieved by developing a technique utilizing label-free surface-enhanced Raman spectroscopy. Each mycotoxin's inherent chemical fingerprint was identified by its distinct Raman spectra, which made it possible to distinguish between them with ease. For corn, the limit of detection (LOD) for AFB1, ZEN, and OTA were 10 ppb (32 nM), 20 ppb (64 nM), and 100 ppb (248 nM), in that order. The SERS spectra of known concentrations were utilized to predict AFB1, ZEN, and OTA concentrations up to 1.5 ppm (4.8 μM) using multivariate statistical analysis; the resulting correlation coefficients were 0.74, 0.89, and 0.72, respectively. Each sample was sampled in less than 30 minutes on average. A potential technique for the quick and simultaneous identification of mycotoxins in maize is the use of label-free SERS and multivariate analysis. This technique may be expanded to include additional mycotoxin kinds and

crops [36]. SERS has been used to check aflatoxins qualitatively with increased sensitivity and specificity by conjugating probe molecules with biomolecular targeting ligands (such as antibodies) and using core-shell nanoparticles. In the standard AFB1 detection procedure, AFB1 is incubated with the SERS aptasensor for a predetermined amount of time, and the resulting complex is then rinsed with buffer solution and re-dissolved in it. The next step is to use a Raman spectrometer to measure SERS by placing many drops on an aluminum plate [24]. Surface-impaired a relatively recent analytical method called Raman scattering has drawn interest as a possible mycotoxin detection substitute. Because surface plasmons are produced when incoming light interacts with a metallic surface, surface plasmon resonance spectroscopy amplifies the Raman signal of molecules adsorbed onto that surface. A broad variety of mycotoxins may be detected by SERS, which has extremely sensitive detection limits in the femtomolar to picomolar range. In addition, it takes less sample preparation time and is non-destructive. SERS has many benefits over conventional analytical techniques for the identification of mycotoxins, such as high sensitivity, low sample preparation requirements, and the ability to detect mycotoxins in situ and in real time [38].

2.2.2 Fourier Transform Infrared Spectroscopy (FTIR)

2.2.2.1 FTIR in food products

The analysis of food components and adulterants has been greatly enhanced by the development of Fourier transform infrared spectroscopy (FTIR) spectroscopy as a potent analytical instrument. Food research has made extensive use of it [39]. The detection of aflatoxin in maize and peanuts has been accomplished with success using Fourier transform infrared spectroscopy [40]. A fast way for determining and measuring aflatoxin contamination in peanuts was devised; utilizing multivariate data processing and Fourier transform infrared spectroscopy. Because this approach is thought to be nondestructive, it was suggested as an alternative to the chromatographic method because it is quicker, more feasible, and requires lesser reagent. With an attenuated total reflectance in the mid-infrared range (4,000–475 cm−1), an FTIR spectrophotometer was used to scan the sample spectra [41]. AFB1 contamination in pure milk was identified and measured using a Fourier transform infrared spectroscopy

in conjunction with multivariate analysis. The AFB1 concentrations of 10, 20, 30, 40, and 50 parts per billion were added to milk, and the FTIR spectra of the contaminated and pure milk samples showed variations in absorption, particularly in the 1800–1331 cm−1 wave number band. The milk samples infected with AFB1 were found to be separated and grouped at the 5% significance level according to principal component analysis. It was determined if it would be possible to detect AFB1 in pure milk using soft independent modeling by class analogies, and the models that were created were able to classify infected samples of milk with certainty [42].

2.2.3 Fluorescence Spectroscopy

One of the most often utilized detection methods in food physical and chemical inspection standards is fluorescence detection. Fluorescence spectroscopy (FS) is another method that may be used to identify aflatoxins like AFM1 and AFM2. The material is generally extracted, centrifuged, filtered, and then run over an immune-affinity column. There, aflatoxin binds with a particular antibody to produce an antibody-antigen complex, which is then passed into a liquid chromatography-fluorescence detection system [43]. The sensitivity can be increased by post-column derivatization with pyridine bromide or iodine; the LOD is around 0.001 µg/kg. Aflatoxin may be found in milk, almonds, animal feed, grain, and oil using this technique [24]. Various fluorescent substances generate energy at distinct wavelengths, making fluorescence spectrophotometry a suitable method for identifying AF. Fluorescence spectrophotometry can quantify AFs in the range of 5 ppb to 5000 ppb in less than 5 minutes [44]. This work examined the use of for the first time in the concurrent detection of aflatoxin B1 (AFB1) contamination in rice and Aspergillus molds using laser-induced fluorescence (LIF) technology. A variety of Aspergillus strains, both toxic and non-toxic, were intentionally injected into rice samples and allowed to develop into different mold and AFB1 infection levels [45]. LIF spectroscopy might be used to track mold activity, according to spectral analysis and principal component analysis (PCA). The kind of infected mold, the degree of mold infection, and the concentration of AFB1 were then used to categorize rice samples using three different classification techniques based on linear and non-linear algorithms. At less than 2%, the greatest accurate classification

rate of 97% was attained. Furthermore, samples of rice flour fared better under discrimination than samples of kernels did. The results of this study confirm that LIF spectroscopy may be used to quickly and non-destructively identify mold and aflatoxin contamination in grains without the need for laborious pre-processing [46].

2.2.4 Near-Infrared Spectroscopy (NIR)

A chemometric framework based on a near-infrared (NIR) spectroscopy approach is designed in this study to quantitatively evaluate aflatoxin B1 (AFB1) in peanuts. With the use of a portable NIR spectrometer, the NIR spectra of peanut samples showing various degrees of fungal infection were recorded [47]. Then, for data refining, the proper pre-processing methods were used. To simplify the study, a preliminary screening of the pre-processed NIR spectra was carried out using the iterative variable subset optimization (IVSO) approach, which removed a large number of unnecessary variables. To enhance this initial screening procedure, the chosen feature variables were further optimized using the beluga whale optimization (BWO) method. Based on the improved near-infrared spectral characteristics, support vector machine (SVM) models were then created to assess AFB1 in peanuts quantitatively. According to the findings, the SVM model considerably enhances generalization ability and detection efficiency, especially following secondary optimization using BWO-IVSO. With a correlation value of 0.9761, a relative percent deviation of 4.6999, and a root mean square error of prediction of 24.6322 μg·kg−1, the SVM model generated following BWO-IVSO optimization demonstrated the most remarkable amount of generalization among the models examined [48]. One major factor contributing to liver cancer in humans is aflatoxin B1 (AFB1). Based on Fourier transform near-infrared (FT-NIR) spectroscopy technology, this work suggests a quantitative detection strategy for the AFB1 in maize. With an FT-NIR spectrometer, we first collected spectrum data on samples of maize with different levels of mildew. Next, we optimized the characteristic wavelengths of the spectra following SNV treatment using the ant colony optimization (ACO) and NSGA-Ⅱ algorithms, respectively. To achieve precise AFB1 detection in corn, back propagation neural network (BPNN) models were ultimately developed utilizing the optimal wavelengths. The resulting data demonstrated that the BPNN model optimized by the

NSGA-Ⅱ algorithm using the four distinct wavelength variables had the best prediction performance. The correlation coefficient of prediction (RP) of the optimal NSGA-Ⅱ-BPNN model is 0.9951, and its root mean square error of prediction (RMSEP) is 1.5606 μg kg−1. Overall, the results show that it is possible to detect AFB1 quantitatively in corn using the FT-NIR technique. Moreover, the NSGA-Ⅱ algorithm offers special benefits for optimizing spectral characteristics, as it can yield characteristic wavelength variables that are both small and highly pertinence [49].

Table 2 Spectroscopic techniques for the detection of aflatoxin in food products

Spectroscopic Detection Techniques	Food Products	Aflatoxin	Chemometric Techniques	Reference
1. Raman	1. Maize	Ochratoxin A, Zearalenone (ZEN), AFB1	Principal Component Analysis (PCA)	[36]
2. Fourier Transform Infrared Spectroscopy (FTIR)	1. Maize 2. Peanut butter 3. Milk	AFB1 AFB1, AFB2 AFM1 AFB1	Principal Component Analysis (PCA)	[40, 42]
3. Florescence Spectroscopy (FS)	1. Rice	AFB1	Principal Component Analysis (PCA)	[46]
4. Near- Infrared Spectroscopy (NIR)	1. Peanuts 2. Maize 3. Corn	AFB1 AFB1 AFB1	-	[48, 49]

2.2.5 Immunochemical Method

2.2.5.1 Enzyme-linked Immunosorbent Assay (ELISA)

The foundation of ELISA is the competitive interactions that occur between mycotoxins, which function as antigens and designated antibodies that are labeled with toxin-enzyme conjugate for several binding sites [22]. The fundamental idea is that an antibody binds to an antigen. The very effective bio-catalytic characteristics of the enzyme and its incredibly precise antigen-antibody recognition serve as the foundation

for this method. After the enzyme and antibody are attached, the color-emitting substrate is transformed into a visible colorimetric output, which is then used to enhance the signal [24].

2.2.5.2 ELISA in food products

Aflatoxin M1 (AFM1), a toxic chemical found in milk, may make it unsafe for consumption. This investigation aimed to evaluate the concentrations of AFM1 in milk, specifically those that surpass the benchmarks established by the European Union (50 ng/L), the Food and Drug Administration (500 ng/L), and the Iranian National Standards Organization (100 ng/L). A total of 180 raw cow's milk samples, 45 from each season, were gathered from several retail dairy markets in Gorgan for the study. The enzyme-linked immunosorbent assay (ELISA) method was used to determine the amount of aflatoxin M1 present in the samples. 139 (72.2%) raw cow milk samples had AFM1 identified in them, ranging from 3.5 to 357 ng/L. The FDA's 500 ng/L maximum limit for aflatoxin M1 concentration was not exceeded in any of the samples that were collected. But when it came to aflatoxin M1 in raw cow's milk, 41 samples (22.7%) went above the EU's 50 ng/L standard and 26 samples (14.4%) over the INSO's 100 ng/L limit. The summer season, which comprised 32 (71.1%) and 38 (84.4%) samples, had the lowest and highest AFM1 levels of contamination, respectively [50]. One of the easiest, fastest, and most popular techniques for identifying aflatoxins is the enzyme-linked immunosorbent assay. The fundamental idea is that an antibody binds to an antigen. The effective bio-catalytic characteristics of the enzyme and its incredibly precise antigen-antibody recognition serve as the foundation for this method [51]. After the enzyme and antibody are attached, the color-emitting substrate is transformed into a visible colorimetric output, which is then used to enhance the signal. The sample was pretreated before being added to the enzyme-linked immunosorbent assay plate containing the immobilized antigen. In the dark, the sample reacted with the enzyme-substrate and antibodies working solution. By evaluating the color-developed solution and stop solution, that were added after the unreacted enzyme and antibodies were rinsed away in the eluent, the color depth of the sample may be used to estimate the quantity of aflatoxin present. Two categories of ELISA exist: direct and indirect. The

antibody is conjugated directly to the enzyme in a direct ELISA. The direct ELISA, in contrast, uses a labeled secondary antibody that is coupled with the detecting enzyme during the main antibody binding process [24]. This research set out to quantify aflatoxin M1 (AFM1) in pasteurized milk from Mashad, northeastern Iran. In order to do this, 42 milk samples were taken from retail establishments in the fall of 2011 and tested using an enzyme-linked immunosorbent assay method for AFM1. Each analysis was conducted twice. Based on an average formulation of 23 ± 16 ppt and a contaminant level from 6 to 71 ppt, the results indicated the detection of AFM1 in 97.6% of the milk samples analyzed. All samples had AFM1 concentrations below the FDA's and the Iranian national standard (500 ppt), with only 3 (1.6%) samples having AFM1 concentrations above the EU's and the Codex Alimentarius Commission's maximum tolerance limit (50 ppt). Based on prior research and our results, AFM1-contaminated milk is not a problem in this area and the regional threshold for AFM1-contaminated milk may be lowered to less than 100 ppt [52]. The most widely used approach for determining mycotoxin immunological techniques is undoubtedly the enzyme-linked immunosorbent test. ELISA offers quick screening, and a variety of commercial kits are available for the detection and measurement of important mycotoxins, such as fumonisins, AFs, AFM1, OTA, ZEA, DON, and T-2 toxins. A large range of food matrices have been used to verify ELISA procedures. The most popular method for doing ELISA is a competitive direct assay, while other methods include direct assay, competitive direct assay, and competitive indirect assay. This method for analyzing mycotoxins in food is quick, precise, and very simple to apply [22]. The reason for this investigation was the identification of aflatoxin B1 (AFB1) in 40 Iranian Tarom rice samples. ELISA, or enzyme-linked immunosorbent assay, was used to examine AFB1 in the specimens. Two analyses of each were performed. In every rice sample, aflatoxin B1 was detected; its concentration varied between 0.29 and 2.92 mg/kg. The 2013 rice samples had a mean AFB1 content that was greater than the 2012 rice samples. But out of the 40 samples, 25 went above the maximum allowed level, which is 2 mg/kg according to EU regulations, and the maximum allowable level was not attained by any of the samples, which is 5 mg/kg according to the Institute of Standards and Industrial Research of Iran (ISIRI) for aflatoxin B1 [53].

Table 3 Immunochemical technique for detecting aflatoxins in foods

Detection techniques	Food Products	Aflatoxin	Reference
1. Enzyme-Linked Immunosorbent Assay (ELISA)	1. Milk 2. Rice	AFM1 AFB1	[24, 53]

Conclusion

Although aflatoxins will always be a problem for public health across the world, every country and area has different difficulties. The problem has grown increasingly complicated as a result of international commerce, climate change, and various regulatory frameworks. To tackle the problem of mycotoxin contamination, several analytical tools are therefore required. Numerous spectroscopic, immunochemical, and chromatographic techniques have been developed to identify aflatoxins. In chromatographic techniques, the HPLC method offers great automation, high sensitivity, and high accuracy in estimating aflatoxins. Rapid, precise, and trustworthy aflatoxin findings are delivered by HPLC in a brief amount of time. However, HPLC-MS/MS is an expensive technology that should only be used by professionals who possess the necessary training and qualifications. Moreover, this limits its application to well-equipped laboratory settings only, excluding outdoor settings. The most rapidly expanding method for analyzing mycotoxins is now the LC-MS approach. Mycotoxin analysis has long recognized and benefited from the potential advantages of the LC-MS approach. A lot of work has gone into quantifying aflatoxins using this method, despite the high initial expenditures of LC-MS equipment. The ability of TLC to detect various mycotoxin kinds with superb resolution and excellent sensitivity is its main benefit. In addition, costly equipment, a qualified technician, and sample pretreatment are needed. Additionally, TLC has several disadvantages that might arise during the production, spotting, and interpretation of TLC plates. In spectroscopic techniques,

detecting molecular vibrations that lead to changes in polarizability which is water-insensitive and produces fewer overlapping bands is clearly advantageous when using Raman spectroscopy. Fast and non-destructive, Raman spectroscopy does not require sample pre-processing for detection. It has been widely employed in a variety of scientific domains and in the quantitative identification of mycotoxins. The benefits of FTIR include high resolution, high radiant flux, minimal stray radiation, and quick scanning. In immunochemical methods, ELISA has the advantage of being an inexpensive, quick approach with low sample quantities and comparatively fewer preparation steps than other methods. It also has good repeatability and repeatability, as well as high specificity and sensitivity. Though its accuracy and repeatability can be increased by having a prior separation step, the nature of the mycotoxin, the sample preparation method, and the material itself can occasionally affect the ELISA's accuracy. These techniques work well for both regulatory and research applications.

Author Contributions

All authors equally contributed for manuscript preparation and editing.

Conflicts of Interest

The authors declare no conflict of interest.

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